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EDITORS :—

Dr C. D. DARLINGTON
John Innes Institution
Bayfordbury, Hertford, Herts.

Sir RONALD FISHER
Whittingehame Lodge
Cambridge

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HEREDITY VOLUME 6 PART 3 DECEMBER 1952

SELF-INCOMPATIBILITY SYSTEMS IN ANGIOSPERMS

I. THEORY

A. J. BATEMAN
John Innes Horticultural Institution
Bayfordbury, Hertford, Herts.

Received 12.iii.52

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I. INTRODUCTION

A SURVEY by Lewis (1944) classified all known incompatible species into two systems:

(i) with multiple allelomorphs; one locus in diploids; sporophytic control of style reaction (with independent action of different alleles) and gametophytic control of the pollen; always homostyled.

(ii) with two alleles per locus; one or two loci; sporophytic control of both pollen and style (alleles showing dominance); with one exception (*Capsella*) heterostyled.

Lewis argued that other combinations of these characteristics would for one reason or another be handicapped and that further research would be unlikely to unearth any new incompatibility system.

On the other hand, Mather (1944) considered that novel systems might yet be found and argued as follows:

"Such a system would presumably use both the multiple allelomorphs of the haplo-diploid and the co-ordination of gene action necessary to impress a somatically determined behaviour on the male gametes in the way shown by the diplo-diploid. It is, however, not yet clear how, or even whether, such a system is a developmental possibility."

Experiments conducted by the author into the genetics of self-incompatibility in the Crucifers *Raphanus sativus* and *Brassica campestris* suggested that these species did not readily fit into an oppositional system such as found in *Nicotiana* by East and Mangelsdorf (1925). Neither were they heterostyled. The literature on other Crucifers previously studied showed anomalous conditions also in *Brassica oleracea*

(Kakizaki, 1930 and Sears, 1937), *Cardamine pratensis* (Correns, 1913; Beatus, 1931) and *Capsella grandiflora* (Riley, 1936).

It seemed desirable therefore, to derive from first principles all conceivable incompatibility systems and to test their ability to function adequately. While engaged on this work papers appeared on incompatibility in two species of Compositae, *Parthenium argentatum* (Gerstel, 1950) and *Crepis foetida* (Hughes and Babcock, 1950). Here not only had the inadequacy of the *Nicotiana* system become apparent but a hitherto undescribed system had been invoked which appeared adequate to account for the data. The need for a return to first principles seemed more urgent than ever.

The highly dispersed incidence of self-incompatibility throughout the Angiosperms is an indication that it has arisen *de novo* a large number of times. (This has been contested by Whitehouse (1950) whose arguments will be considered later.) In which case it would be only to be expected that all incompatibility systems (just as all floral mechanisms promoting cross-pollination) which could withstand the rigours of natural selection and which were physiologically possible might be encountered somewhere among the families of flowering plants.

2. MINIMUM REQUIREMENTS OF AN INCOMPATIBILITY SYSTEM

The primary function of self-incompatibility is the avoidance of self-fertilisation. It differs from all other mechanisms such as protandry, protogyny, monoecy, dioecy and all special floral mechanisms which achieve the same end more or less effectively, in that while all the rest do so by interposing some *mechanical barrier to self-pollination* self-incompatibility acts at a later stage, by interposing a *physiological barrier between self-pollination and self-fertilisation.*

In *Oenothera organensis* (Emerson, 1938) the difference in growth of compatible and incompatible pollen tubes is very marked. Compatible tubes rapidly grow down the very long style, while incompatible tubes do not grow long enough to empty the pollen grain. Such a rigorous inhibition of self-pollen is not necessary, however, for self-fertilisation to be suppressed under natural conditions. Except when the pollinator (wind or insects) is excluded there will be enough compatible pollen on the stigma to grow down the style before self-pollen unless the growth rate of self-pollen tubes approaches closely to that of fully compatible pollen or unless self-pollination occurs in the bud. Even when self-pollen tubes grow fast enough for self-fertilisation under natural conditions to be a common occurrence the degree of cross-fertilisation may still be enough for the requirements of the species. No one doubts the selective value of protandry and monoecy which only partially reduce self-fertilisation. The natural frequency of partially self-fertile incompatible species is high. This can be seen from the literature in the number of experimenters on self-incompatibility whose work has been hampered by pseudo-compatibility. Even an incompatibility system which acted in such a way that a proportion of

the individuals were fully self-fertile might be favoured by natural selection. As an outbreeding mechanism it would be comparable in efficiency to the widespread gynodioecy developed in the Labiatae and Caryophyllaceae.

Whatever the details of any genetically determined incompatibility system, there must always be cross-incompatibility between genetically similar individuals. Such cross-incompatibility is only an outbreeding mechanism when it reduces the chances of sib mating relative to mating with non-sibs. In dimorphic heterostyled species the cross-incompatibility can have no selective value because the proportion of the two mating types among sibs (1:1) is the same as in the whole population. Such cross-incompatibility must be merely a fortuitous by-product of the breeding mechanism.

In a species like *Nicotiana sanderae* with a large number of oppositional alleles, cross-incompatibility between unrelated individuals will be negligible. The progeny of two individuals with no common allele falls into four cross-compatible classes though only half the *pollen* of sibs (owing to half-compatibility between plants with one common allele) will be compatible. Relative to the general population the chances of sib mating would, therefore, be reduced by a half. This will have a much smaller effect on the degree of outbreeding of the species than the complete inhibition of self-fertilisation. It is unlikely, therefore, that the effect of any particular incompatibility system on cross-incompatibility will play an important role in determining its overall selective value. Mather (1944) also holds that in flowering plants its effect on sib mating is likely to be a trivial consideration in determining the selective value of a mating system.

3. COMPLEMENTARY AND OPPOSITIONAL SYSTEMS

There would seem to be two basic ways of determining self-incompatibility, the stimulation of unlike genotypes and the inhibition of like genotypes. These may be distinguished by the terms complementary and oppositional, the latter being East's original term.

A complementary system involves the mutual dependence of a pair of genotypes for the completion of sexual reproduction. In this way, male and female individuals in dioecious species are complementary though here not only the physiology, but the morphology also are complementary. Something closer to the incompatibility of higher plants is encountered in heterothallic Mucorales in which the presence of the complementary mating type stimulates the development of sex organs. Moewus' account (1939) of heterothally in *Chlamydomonas* also indicates a complementary relationship between mating types. Some of the heterostyled flowering plants appear to have a complementary system, as for example, *Linum grandiflorum* (Lewis, 1943*a*) and *Forsythia intermedia* (Moewus, 1950).

A mechanical model of the complementary relationship could be given by lock and key, the key of the pollen of one mating group fitting

the lock of the other. Such a model emphasises the limitations to the number of possible complementary mating types. Even with three types, each type must carry with it the " keys " to the " locks " of the two others. It is difficult at first sight to see how even a three-type complementary system could work, but the morphological arrangement in tristylic *Lythrum salicaria* provides the clue. Each form has one style length (one lock) and two anther heights (two keys). As each anther height from whichever form is only compatible on one style the parallel with the " key and lock " theory is complete. Each anther height corresponds to a single " key " so that each form carries two " keys " by virtue of its two anther levels. Apparently both " keys " cannot be borne in the same anther and one " key " cannot fit two " locks ". The principle is illustrated in fig. 1.

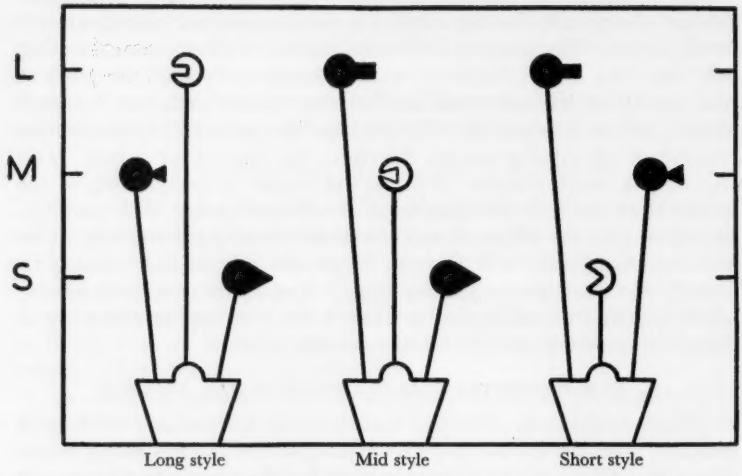


FIG. 1.—Symbolic representation of a complementary incompatibility mechanism in a trimorphic heterostyled species. White circle=stigma; black circle=anther.

Oppositional systems depend upon an antagonism between style and pollen of like genotypes. East (1929) was the first to postulate the antigen-anti-body reaction to explain this, the pollen producing the antigen and the style the anti-body. No direct evidence for the correctness of this hypothesis has so far been published, but in the asbence of a better hypothesis we are justified in using it as the underlying assumption in our discussion of oppositional systems.[1]

The most important distinction between the reactive compounds responsible for incompatibility and the antigen-anti-body system of serology is that the antigen does not induce the production of the anti-body; *both* are preformed. In this they are more like the A B O blood groups in man in which O blood contains anti- A and anti- B before the addition of the A and B antigens. Also one cannot objectively

decide in the case of incompatibility which would be the antigen and which the anti-body. Since, however, it is the pollen whose growth is inhibited, we may take it as a working hypothesis that the pollen produces the antigen which is coagulated by the stylar anti-body.

That there is a positive antagonism between style and self-pollen has been shown by Lewis (1942) in *Prunus*, *Oenothera* and *Primula*. The growth of compatible pollen was greater at high than at low temperature, but incompatible pollen grew faster at the low temperatures.

The present classification of incompatibility systems cuts across previous ones such as hetero- *versus* homomorphic (*e.g.* Lewis, 1944) or haplo-diploid *versus* diplo-diploid (Mather, 1944). The author regards this distinction between complementary and oppositional systems as the fundamental one. The relations between different methods of classi-

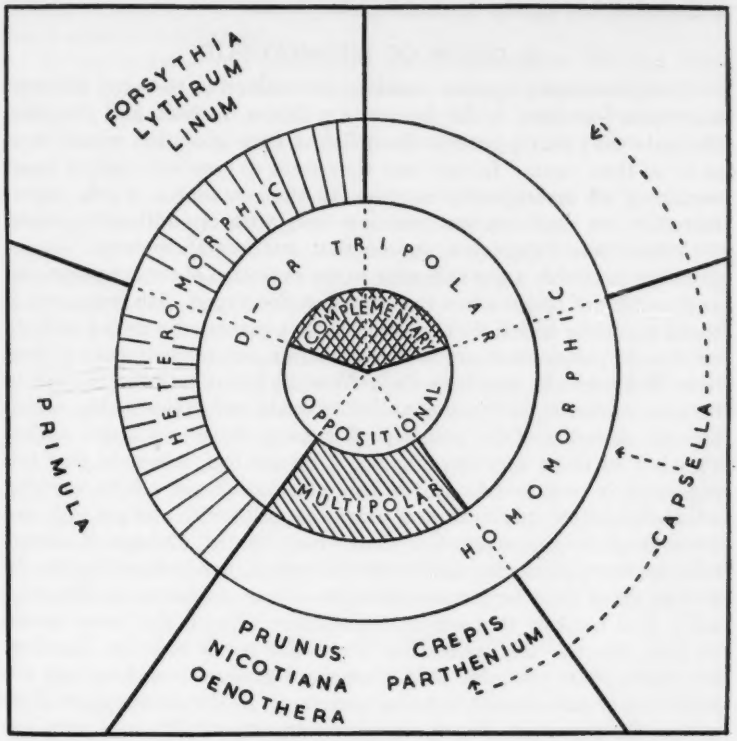


FIG. 2.—A comparison of three classifications of incompatibility systems.

fication are shown in fig. 2 with a few examples. Mather's haplo-diploid versus diplo-diploid classification is omitted here as apart from heteromorphic systems where the determination is probably always diplo-diploid, either of his classes could work in conjunction with any other combination of classes.

If the combinations of the three pairs of classes were random, there would be 8 different combinations. However, heteromorphism and complementary relations of the mating groups both exclude multipolar systems so that only five combinations are theoretically possible. Examples of three amongst flowering plants are well established: complementary, di- or tripolar, heteromorphic; oppositional, dipolar, heteromorphic; and oppositional, multipolar, homomorphic. The combination of tripolar and homomorphic is only known in the apparently anomalous species *Capsella grandiflora*. Here the close genetical similarity to *Lythrum* indicates the possibility of a complementary system, but the situation in Capsella will be discussed at length in a second paper. There are no other examples of Angiosperms in either of the remaining combinations though *Mucor* is an example among cryptogams of a complementary dipolar homomorphic system.

4. ORIGIN OF INCOMPATIBILITY

Complementary systems could be determined in so many different ways (the few cases so far known, *e.g. Linum*, *Lythrum* and *Forsythia*, illustrate this) that a general discussion of their evolution would seem to be of little value. In any case they seem to represent only a small section of all incompatible species. In the remainder of this paper, therefore, we shall concern ourselves only with oppositional systems.

Whitehouse (1950) has argued that multiple-allelomorph incompatibility probably arose only once in the evolution of the Angiosperms, at the point of origin when the style first developed. His argument is based upon the beliefs that (*a*) the selective powers the style is able to exert upon pollen tubes are its main function and (*b*) that since at least three **S**-alleles are necessary for a *Nicotiana* incompatibility system to become workable, such incompatibility could only arise by the simultaneous mutation of the prototype **S**-gene to three new active alleles. This is held to be very improbable. I do not feel, however, that this argument is very sound. In the first place to obtain a fully working self-incompatible species from a previously self-fertile one not only are three **S**-alleles necessary, but there must be the division of labour between style, producing anti-body and pollen, producing antigen. In a word there must be the evolution of a large number of modifiers as well. It is unlikely that self-incompatibility when it first arose would be fully effective any more than any other newly evolving function, but much more probable that it emerged gradually without any coincidence of rare mutations being necessary. In the second place if we accept his argument that the origin of incompatibility is a very improbable event, how much less likely is it to arise among the few incipient Angiosperm individuals at the point of origin of the phylum than among the enormous number of Angiosperms living to-day?

Whitehouse further bases his argument on the assumption that the absence of a style in Gymnosperms precludes self-incompatibility based on a stylar reaction. Reference to Coulter and Chamberlain (1910)

shows that it is typical of gymnosperms for pollen tubes to be compelled to grow through a well-developed nucellus before fertilisation is possible. Since in *Capsella* (Riley, 1936) and *Parthenium* (Gerstel and Riner, 1950) a single layer of cells in the stigma is sufficient to inhibit self-pollen, the massive nucellus of most gymnosperms should be quite capable of acting as a physiological sieve for pollen tubes. The absence of a style in gymnosperms is not, therefore, a sufficient explanation of the absence of self-incompatibility from this phylum (if it is indeed absent).[2]

We shall now propose an alternative theory of the evolution of incompatibility. Let us consider a species in which there is a rather loosely operating outbreeding mechanism, such as a morphological adaptation of the flowers conducive to cross-pollination and let us assume that the environment is one in which more rigorous outbreeding has a selective advantage.

In any outbreeding population, many phenotypes differing serologically may co-exist. This is known for example in man (Race and Sanger, 1950) and cattle (Owen *et al.*, 1945) and it might be expected that similar situations would hold in plants though they have not yet been found.

In man it is known that several loci are involved in this variation. The large number of serological types in cattle points to a similar conclusion. It is not illogical to suppose that genetically heterogeneous plant populations would also vary serologically in a similar way. With such raw material already available all that would be needed to produce a primitive oppositional incompatibility system is a division of labour so that the style produced anti-bodies to those antigens in the pollen for which the population was heterogeneous. The production of anti-bodies to antigens for which the population was homogeneous would merely make the plant female-sterile. This anti-body production would, therefore, have to have a measure of specificity from the start, but if, at the time of origin of the incompatibility the population was heterogeneous for several antigens there could be several "**S**-loci", each locus producing alternative pairs of antigens (and anti-bodies). Allowing for the qualitative differences between all incompatibility alleles and loci, this is a close approach to a typical polygenic system.

5. DEVELOPMENT OF INCOMPATIBILITY

At the time of origin the incompatibility would probably not be at full strength though even then it might be adequate for the requirements of the species. In which case one might expect to find species which had stabilised at this evolutionary level. Characteristic of such species would be a high degree of pseudo-compatibility with nevertheless consistently better setting on crossing than on selfing. If several antigen loci were involved, compatibility between sibs would vary according to the number of common alleles, but would be intermediate between selfing and crossing with unrelated plants. Further analysis

of such species would be difficult because of the psuedo-compatibility.

If, however, such a weak system were inadequate for the requirements of the species it could be improved upon in two ways, by the selection of non-specific modifiers increasing the effectiveness of all loci, or of specific modifiers increasing the effectiveness of one or two loci at the expense of the rest.

The first could proceed as far as complete incompatibility dependent on the additive effects of all the loci or complete incompatibility produced by correspondence at any one locus. Where all loci had to act simultaneously cross-compatibility might vary in a graded series according to the number of common antigens. If, however, there were (as might well be) a critical threshold strength of the reaction below which all pollinations appeared fully compatible, identity in more than a critical number of antigens would give incompatibility, less than that, compatibility. The results in such a species would probably be difficult to interpret by any clear-cut scheme especially if different loci varied in the strength of their reactions.

The simplest case would be when all the loci acted independently, additively and with equal strength, identity at all loci being necessary for full incompatibility. Cross-compatibility between sibs from a cross between parents with two active loci only and no alleles in common would be as follows. There would be 16 genotypes and out of every 16 cross-pollinations:

With gametophytic control of pollen there would be:

9 fully compatible, at least one quarter of the pollen having no allele in common with the style.

6 partially compatible, at least one quarter of the pollen having only one allele in common with the style.

1 incompatible.

With sporophytic control of the pollen and independent action of the alleles there would be:

1 pollination fully cross-compatible (no common allele).

4 highly cross-compatible (1 common allele).

6 half compatible (2 common alleles).

4 weakly incompatible (3 common alleles).

1 fully incompatible (4 common alleles).

With more than two loci an even more finely graded series of compatibilities would be possible.

Selection of non-specific modifiers could reach the point where identity at a single locus was sufficient for full incompatibility. Cross-compatibility between sibs would be increasingly rare as selection proceeded or with increasing numbers of active loci. With gametophytic control of pollen and n loci the proportion of cross-incompatibility between sibs whose parents had no allele in common would be

$$\frac{1+\frac{3}{4}+\left(\frac{3}{4}\right)^2+\cdots\left(\frac{3}{4}\right)^{n-1}}{4}.$$

With 8 loci it would be 0·900. With sporophytic control and independence it would rise much more rapidly.

This situation resembles that in polyploids except that since in that case the loci are duplicated it is possible for the same allele to occur at either locus so that the incompatibility reaction could be exerted between different loci. This would, however, be expected to be rare except in recent autotetraploids or in species where the number of possible alleles was low. The evidence so far is that except in heteromorphic species the number of alleles is very high indeed.

The second way of improving the efficiency of a polygenic system with initially incomplete efficiency of each locus would be the selection of specific modifiers acting upon one or perhaps two loci. This is the type of incompatibility hitherto assumed to be generally applicable. An outwardly similar result would be obtained if the several active loci, as a result of translocations and inversions, came to be within a single tight linkage group. Unless, however, complete identity of all the units in such a " locus " was necessary for full incompatibility there would be cross-incompatibility between non-identical compound " alleles ".

6. VARIATIONS ON THE OLIGOGENIC THEME

Oligogenic incompatibility is not only the one generally recognised: it is probably in fact the most widespread, the multigenic systems discussed above being intermediate stages in the evolution of fully developed oligogenic systems. It is also the simplest type theoretically and, therefore, the most suitable medium for a discussion of further modifications in the detailed working of the incompatibility reaction.

The possible alternatives, sporophytic and gametophytic (or diploid and haploid) control have already been mentioned. Although the pollen is haploid not all the behaviour of the pollen need necessarily be determined by its own haploid nucleus. Much of the cytoplasm and possibly the entire wall of the pollen grain will have been determined sporophytically, by the constitution of the pollen mother cells or even the tapetal cells. The reaction of the style must be diploid. It is just conceivable, however, that in the absence of a style reaction the embryo sacs themselves might be able to exert an inhibitory influence on pollen tubes of like constitution.

When there is gametophytic control in a diploid species there is no possibility of interaction between pairs of **S**-alleles because they will always occur singly. But as Lewis has shown (1947), in tetraploid species with diploid pollen there is such a possibility and, moreover, it exists. Just the same possibilities arise in diploid species wherever there is sporophytic control in style or pollen. Lewis found in tetraploid *Oenothera* that the interaction could manifest itself in two ways which he called competition and dominance. With competition both alleles were weakened; with dominance only one was inactive. Since the dominance could equally well be regarded as the result of competition

in which the weaker allele was suppressed, I prefer to use the more explicit terms mutual weakening and dominance.

In *Oenothera* the mutual weakening in heterozygous pollen grains, while increasing the tube length in self-pollinated styles did not lead to self-fertility. In other species such as *Trifolium repens* (Atwood, 1944) and *Pyrus communis* (Crane and Thomas, 1939) self-fertility is produced. In diploid species with sporophytic control of the pollen, whether the weakening would result in self-fertility would depend upon its selective value. If such self-fertility were undesirable to the species it could easily be eliminated by natural selection, either by reducing the threshold of the inhibition necessary to produce incompatibility or by increasing the amount of substrate the competition for which has led to the weakening.

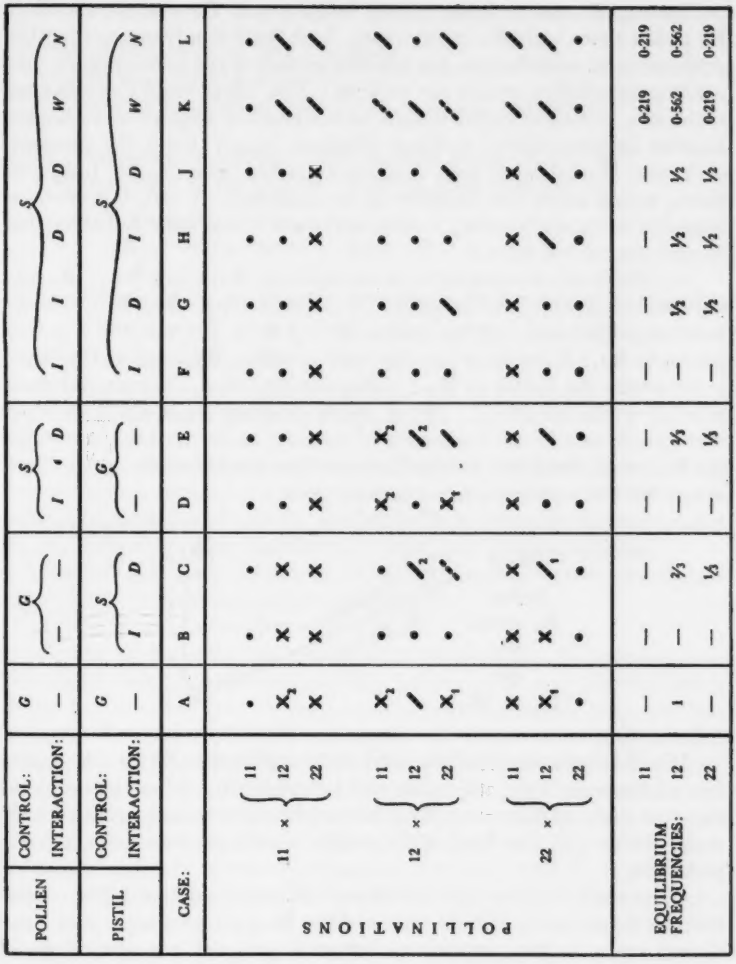
A third type of interaction between **S**-alleles, would be the production in heterozygotes of a new antigen, distinct from that of the homozygotes. From our knowledge of gene action this would seem unlikely, but as such a situation has been considered by Lewis (1943*b*) and has been even postulated by Fernandes (1935) in *Narcissus triandrus*, it has been included for completeness.

Table 1 lists all possible combinations of gametophytic and sporophytic control with independence or dominance in pollen or style, but with the simplest background: one locus with two alleles. When there is dominance it is assumed to be of $\mathbf{S}_1$ over $\mathbf{S}_2$. There are three possible genotypes, represented for simplicity without the **S**'s as 11, 12 and 22, with 9 possible pollinations. All compatible pollinations are represented by a stroke, a heavy one when fully effective. When only one of the two kinds of gametes functions, this is shown by a suffix. When either of the compatible genotypes will be eliminated after one generation the stroke is crossed through. When the seed parent of a compatible cross-pollination is self-fertile it is assumed (except in E) that such a pollination is unimportant genetically and the stroke is broken. In case K the two compatible back-crosses together produce the same results as self-fertilisation so that even if they were frequent, they would have no effect on the frequencies of the three genotypes. Only in C would cross-fertilisation of the heterozygote produce a different result from the assumed complete self-fertilisation.

Three of the cases B, D and F are unworkable at the one locus two alleles level, for all compatible pollinations involve a genotype which could not exist for more than one generation. That this has no bearing on their practicability with more alleles is shown by the fact that B represents the *Nicotiania* type of incompatibility and F that frequently found in *Crepis* and *Parthenium*.

Let us now consider the workable cases individually. It is necessary to pause here to discuss gametophytic control on the female side, which does not seem to have been considered hitherto. If the embryo sacs secreted anti-bodies into the carpellar tissue and these diffused up the style the results would be indistinguishable from sporophytic control.

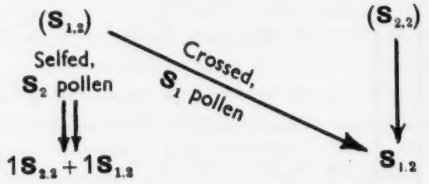
TABLE 1



Possible oppositional incompatibility systems with 1 locus and 2 alleles. At head of columns the letters *G* and *S* stand for gametophytic and sporophytic control. With sporophytic control there are four alternative interactions between two alleles in the heterozygous state: independence (*I*), dominance (*D*) of $\mathbf{S}_1$ over $\mathbf{S}_2$, natural weakening (*W*) and an entirely new action (*N*). The three genotypes are represented by the suffixes to the **S** symbol alone. For explanation of the body of the table see text.

It is just conceivable, however, that specific inhibitory substances could be secreted by ovules which had a very small radius of action. Growth of incompatible pollen tubes would be normal up to its inhibition at a point just prior to fertilisation. In this way, in A when $S_{1\cdot2}$ was self-pollinated, S_1 pollen tubes would fertilise only S_2 embryo sacs and S_2 pollen tubes, only S_1 embryo sacs. In A there is only one compatible pollination at equilibrium, the self-fertilisation of the heterozygote. No self-incompatibility would be evident. The effect would correspond more to a balanced lethal system without loss of zygotes or to haplo-haploid incompatibility in fungi (Mather, 1944). Only the presence of further **S**-alleles, all with dominant marker genes closely linked to them, would allow the situation to be analysed. It can, however, be regarded as an outbreeding mechanism since it maintains heterozygosis at and near the **S**-locus.[3]

C. There are two genotypes at equilibrium, $S_{1.2}$ and $S_{2.2}$. $S_{1.2}$ is self-fertile with only half the pollen (S_2) functioning. $S_{2.2}$ is self-sterile but compatible with half the pollen (S_1) of $S_{1.2}$ If crossed as a pollen parent to $S_{1.2}$ it produces just the same as selfing $S_{1.2}$ but as the latter is self-fertile the pollen of $S_{2.2}$ will rarely function. The equilibrium position would be $2S_{1.2} : 1S_{2.2}$. Such a system could easily develop into gynodioecy by accumulation of recessive male-sterility genes near the S_2 locus. Similarly, deleterious recessives would rapidly accumulate at the S_1 locus which is never homozygous.



It will be seen that half the seed of the self-fertile will be self-sterile, but all the seed of the self-sterile will be self-fertile. After the development of male sterility, the $S_{1.2}$ hermaphrodites would produce half male steriles and the $S_{2.2}$ male steriles would produce only hermaphrodites.

Once male sterility had developed the self-incompatibility would become superfluous. If it disappeared the $S_{1.2}$ ♀ on selfing would produce ♀ and ♀ in the proportions between 3:1 and 2:1 according to the viability of $S_{1.1}$ and the ♀ would produce ♀ and ♀ in the proportion 1:1. This would alter the equilibrium frequencies however. If $S_{1.1}$ were inviable the equilibrium would become 3 : 2 (an increase in the frequency of ♀). If $S_{1.1}$ were fully viable the ♀ would be eliminated, as Lewis has shown (1941), and all trace of an outbreeding mechanism would have disappeared. Case C, with or without derived male sterility, would be just as effective an outbreeding mechanism as

male sterility derived independently of incompatibility. The possible evolution of C is shown below in chronological order:

1. Self-incompatibility with 2 alleles **S**1 and **S**2:
   S1 dominant over **S**2 in style: gametophytic control of pollen.

   Seed of **S**1.2 will be 1**S**1.2 : 1**S**2.2.
   Seed of **S**2.2 will be **S**1.2 only:
   Equilibrium: 2**S**1.2 (self-fertile) : 1**S**2.2 (self-sterile).

2. Accumulation of recessive male sterility genes close to **S**2:
   Accumulation of deleterious recessives close to **S**1.

   Equilibrium (as in stage 1):
   2**S**1.2 (self-fertile ♀) : 1**S**2.2. (♀)

3. Breakdown of incompatibility reaction with **S**1 acting as recessive lethal:

   Seed of **S**1.2 will be 2**S**1.2 : 1**S**2.2.
   Seed of **S**2.2 will be 1**S**1.2 : 1**S**2.2.
   Equilibrium: 3**S**1.2 ♀ : 2**S**2.2 ♀.

Lewis' argument (*ibid.*) against the likelihood of naturally occurring recessive male sterility could be circumvented, therefore, if it arose via self-incompatibility of case C. Evidence for its origin in this way would be the lethality or low viability of homozygous hermaphrodites.

Cases D and E with the control of the pollen and style reversed yield situations corresponding closely to B and C.

D, like B, is unworkable with 2 alleles.

E, like C, has only two genotypes at equilibrium, the homozygous dominant being eliminated and the homozygous recessive having a frequency of one-third. A very unusual situation arises from the fact that the **S**1 ovules of the heterozygote will reject all its pollen tubes which will be phenotypically **S**1 owing to dominance. Consequently only the **S**2 ovules will be fertilised on self-pollination (giving **S**1.2 and **S**2.2 seed in equal frequencies) and the **S**1 ovules will remain available for **S**2 pollen from the homozygotes. We thus have self-fertile plants in which with free access of pollinators, regularly half the seed will be self-fertilised and half cross-fertilised. The pollen of the homozygotes will thus have a function to perform and male sterility would be unlikely to arise.

We now consider those cases in which control is sporophytic in both style and pollen. As already stated, F is unworkable with only 2 alleles.

G has only one compatible pollination **S**1.2 × **S**2.2. Thus neither the pollen of **S**1.2 nor the style of **S**2.2 is functioning. We have a hermaphrodite morphology but functional dioecy with **S**1.2 acting as female and **S**2.2 as male. Such a system would not be expected to function long without evolving into morphological dioecy by loss muta-

tion, the **S**-bearing chromosome acting as sex-chromosome and the females being heterogametic. Thus we see a possible path for the evolution of sex through incompatibility.

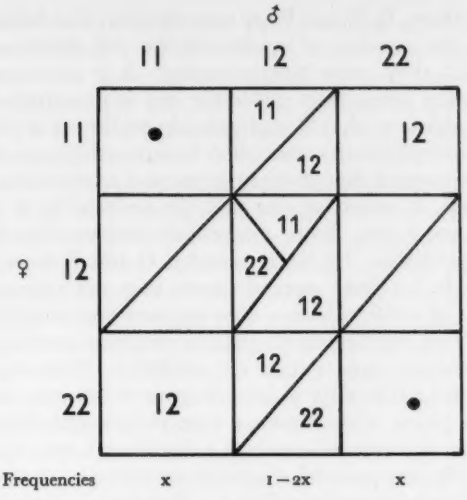
H. In just the same way and for the same reasons this would be expected to evolve into dioecy with heterogametic males.

J. Here there are two groups again but this time they are cross-compatible in either direction. As a breeding mechanism it is identical with the dipolar heterostyly of *Primula*. $\mathbf{S}_1$ corresponds to **S** and $\mathbf{S}_2$ to **s**. The heterostyly is, however, lacking. Since, however, half the pollinations would be incompatible there would be a large wastage of pollen which could be overcome by a morphological mechanism which reduced the chances of incompatible pollinations. Heterostyly as in *Primula* may be such a mechanism, though I am not aware that it has been proved experimentally. In which case selection might favour the evolution of heterostyly from such a homostyled incompatibility. On the other hand in *Armeria* and *Limonium* (Baker, 1948) the dimorphism is not of a kind likely to reduce the chances of illegitimate pollination. It might be directly related to the physiology of the incompatibility itself, whether oppositional or complementary.

K represents the weakening of both alleles in the heterozygous condition, in both style and sporophytically controlled pollen. One could have considered unilateral weakening in combination with dominance, independence and gametophytic control, but this would have led to unnecessary elaboration of the argument, since whether the weakening is in pollen, style or both, the main effect will be the same, namely, self-fertility of the heterozygote together with self-sterliity of the homozygotes, leading to a continuous cycle from self-fertilisation to cross-fertilisation with each generation. Within a single population this provides a situation in which at the same time there will be much of both potential and free variability. The potential variability will reside in the plants produced by cross-fertilisation of the self-steriles. The free variability will arise by the self-fertilisation of the self-fertiles produced by cross-fertilisation. Such a breeding system might have special advantages when a rapid and large scale response to selection was required.

To consider K in detail. This is best done with the aid of the mating diagram opposite (symbols as in fig. 3).

The progenies obtained from each compatible mating are shown in the appropriate squares. At generation n the frequencies of $\mathbf{S}_{1.1}$ and $\mathbf{S}_{2.2}$ will be equal, say x. Then the frequency of $\mathbf{S}_{1.2}$ is $(1-2x)$. In calculating the equilibrium frequency it is assumed that all genotypes are equally viable and fully fertile whether self-fertile or self-sterile. The contribution of a genotype as seed parent to the next generation will, therefore, be in proportion to its frequency in the general population. Owing to cross-incompatibility, however, the contribution of a genotype as pollen parent to the progeny of any seed parent will be in proportion to its contribution to the pool of *compatible pollen*, which will not normally be the same as its frequency in the general population.



On these assumptions if x_{n+1} is the frequency of **S**1.1 at generation $(n+1)$

$$x_{n+1}=x_n\cdot\tfrac{1}{2}\cdot\frac{1-2x_n}{1-x_n}+(1-2x_n)\cdot\tfrac{1}{4}$$
$$=\frac{1-x_n-2x_n^2}{4(1-x_n)}$$

At equilibrium $x_{n+1}=x_n$. This gives us a quadratic equation in x, of which the solutions are 2·28075 and 0·21925, of which only the second is real. Therefore the equilibrium frequencies are, for **S**1.1 and **S**2.2, 0·219 and for **S**1.2, 0·562. If we allow for cross-fertilisation of **S**1.2 by **S**1.1 and **S**2.2, the two back-crosses together, being equally frequent, will yield the same genotype in the same ratios as **S**1.2 selfed. The equilibrium would, therefore, be unaffected by cross-fertilisation of the self-fertile plants.

L. This involves a rather hypothetical type of gene reaction. The heterozygote is supposed to produce a reaction of equal strength to either homozygote, but qualitatively distinct. The only reason for its inclusion is that it has been proposed by Fernandes (1935) to explain the inheritance of tristyly in *Narcissus triandrus*. It is, however, of interest to students of population genetics since it represents an example of complete genotypically dissortative mating. Each genotype is compatible only with the other two genotypes. In spite, however, of the novel determination the equilibrium is exactly the same as in K with complete self-fertilisation of the heterozygotes, namely 0·219 for **S**1.1 and **S**2.2 and 0·562 for **S**1.2.

Summarising the discussion of these eleven distinct cases involving an oppositionally determined self-incompatibility with two alleles at

one locus, only three, B, D and F are unworkable. The failure of these three is due to the presence of too few alleles. All the remainder are workable in that they are self-perpetuating. A is unrecognisable as self-incompatibility being fully self-fertile but is nevertheless an outbreeding mechanism in that it maintains hybridity at a given locus. To date no gametophytically controlled incompatibility on the female side has been discovered though there seems no *a priori* reason for ruling it out altogether. It would appear that the probability of finding A, D and E in nature is low. From theoretical considerations L must be regarded as improbable. Of the remainder, C and K have self-fertile heterozygotes. It has been argued above that the occurrence of a regular amount of self-fertilisation does not undermine self-incompatibility and may even supplement it, giving a resultant breeding mechanism with particularly rapid release of variability. There is, however, one reason against self-fertility in heterozygotes being very widespread. In doubled-up plants with *Nicotiana* type of incompatibility heterozygous pollen is not always self-fertile (*e.g. Oenothera*, Lewis, 1947). Only certain pairs of **S**-alleles would, therefore, be expected to show mutual weakening in heterozygous conditions. However, a certain amount of self-fertility is common in otherwise self-sterile species and much material for studies in self-incompatibility is consciously selected for absence in self-fertility. Whether or not self-fertility in heterozygotes is a systematic feature of any self-sterile species, some of the sporadic self-fertility so frequently encountered (in particular found by Kakizaki, 1930, in *Brassica*) may be attributable to this effect.

C, G, H and J produce conditions appropriate to the evolution of gynodioecy, sex and heterostyly. Except in the last named the original incompatibility may become submerged, leaving little, if any trace of the path along which the later condition was derived.

7. SEVERAL ALLELES

Having discussed the simplest possible situation (one locus with two alleles), it is necessary to pursue the matter further by considering the action of several alleles. The main effect naturally will be an increase in the number of cross-compatible groups. Consequently where two alleles were inadequate to give any cross-compatibility (B, D and F of table 1) the systems become workable.

B. Three alleles will provide three cross-compatible mating groups; **S**1.2, **S**1.3 and **S**2.3. The cross-compatibility within the population rises from zero to 2/3. This is, of course, the *Nicotiana* system. But in all species so far studied there has always been a large or very large number of **S**-alleles. Once a species had achieved several alleles the cross-compatibility would be so high that it is doubtful whether any further increase would be advantageous to the *species*. However, any new allele will always have an initial advantage over its predecessors, in the first place because if it arises in a pollen grain that grain will be com-

patible with its own style, and in the second place because until it reaches equilibrium it will always have a lower amount of cross-incompatibility to meet than the older alleles. It is a feature of this scheme that any one genotype can never arise as seed on a plant of the same genotype. This has a balancing effect tending to keep all genotypes equally frequent.

It may be noted in passing that this selection towards the equal frequency of all alleles and genotypes weakens Fisher's criticism (1947) of the author's estimates of the number of **S**-alleles in clover populations (Bateman, 1947). The criticism was that extreme variation in gene frequencies would cause under-estimation of the total number of alleles. Such extreme variation could only arise through mixing of populations or mutation and would be eliminated by natural selection.

D. The advent of a third allele would immediately produce a workable system as in B. New alleles would again be favoured by selection. One distinguishing feature between B and D is that in matings between plants with one common allele B will give a full seed set producing the paternal class plus a third, whereas D will give a 50 per cent seed set producing the maternal class plus a third.

F. Even three alleles does not give a workable system, for all homozygotes are eliminated at once and the three heterozygotes, **S**1.2, **S**1.3 and **S**2.3 will be cross-incompatible since any pair has one common allele. With four alleles there will be six heterozygotes and each of these will be compatible only with one other, that with no common allele. Hence cross-compatibility within such a population would be only one sixth. It is doubtful whether fertility of the plants would be adequate for survival. With five alleles there is considerable improvement. There will be ten classes with cross-compatibility 3/10. Six alleles gives 15 classes with cross-compatibility of 2/5. Even this is lower than B or D with 3 alleles.

Thus under sporophytic control with independence of the alleles, the species could not survive with less than four alleles and the general fertility, depending on cross-compatibility, would be low even with more alleles. There would be strong selection favouring a large number of alleles, or, alternatively, non-independence. This suggests why *Parthenium* and *Crepis*, recently found to have completely sporophytic control, show considerable non-independence.

An interesting corollary to such a system is that since compatible pairs cannot have a common allele there is only one kind of family, falling into 4 classes with a cross-compatibility between sibs of one quarter. When there are only four alleles this is actually higher than the cross-compatibility in the entire population (one sixth). Under these circumstances *sib mating, far from being discouraged by self-incompatibility, would actually be promoted.*

Let us now consider those cases which already worked with two alleles.

A. As all heterozygotes are self-fertile and breed true, with three

alleles the population would consist of three independent lines whose frequencies would be independent also. Crossing two lines would produce an unusual result. With $\mathbf{S}1.2 \times \mathbf{S}1.3$ the S1 ovules would be fertilised by $\mathbf{S}3$ pollen only, whereas the $\mathbf{S}2$ ovules would accept $\mathbf{S}1$ and $\mathbf{S}3$. The progeny, therefore, would be $2\mathbf{S}1.3$ (paternal) : $1\mathbf{S}1.2$ (maternal) : $1\mathbf{S}2.3$ (new genotype). None of these types would be distinguished by breeding behaviour since they are all self- and cross-fertile. The only possibility of recognising such a system would be by the recognition of dominant markers linked to the various **S**-alleles.

L. The very special interaction between the **S**-alleles in heterozygotes would make it appear even less likely that a third allele with a similar interaction with both the preceding ones could arise.

All the other cases involve dominance or weakening in heterozygotes. If, therefore, the number of alleles increases beyond two, the number of possible combinations and permutations of dominance, independence, or weakening between the different pairs of alleles becomes very numerous. Let us illustrate this by taking the two relations dominance and independence and making the arbitrary assumption that with three alleles $\mathbf{S}1$, $\mathbf{S}2$ and $\mathbf{S}3$ only dominance of a lower number over a higher can occur (this amounts to predetermining the symbols we may use for the three alleles). It also implies that if A is dominant over B and C, C cannot be dominant over A. If $>$ is used for dominance of left over right and $=$ for independence, then we may have:

$$\begin{aligned} &\mathbf{S}1 > \mathbf{S}2 > \mathbf{S}3, \\ &\mathbf{S}1 > \mathbf{S}2 = \mathbf{S}3, \\ &\mathbf{S}1 = \mathbf{S}2 > \mathbf{S}3, \\ \text{or } &\mathbf{S}1 = \mathbf{S}2 = \mathbf{S}3. \end{aligned}$$

It must also be recognised that the incidence of dominance in pollen and style can be largely independent (Gerstel, 1950, and Hughes and Babcock, 1950). There are, therefore, 16 possible combinations of pollen and style reaction excluding any account of weakening in heterozygotes. One of these includes complete independence as described above under F. This leaves 15 combinations of sporophytic control, which together with three with gametophytic control of pollen and three with similar control of ovules makes 21 combinations for us to consider. All would be expected to give slightly different equilibrium values for the various genotypes.

We shall, therefore, consider only five representative samples. We shall assume that dominance, if it occurs, is in the form $\mathbf{S}1 > \mathbf{S}2 > \mathbf{S}3$ and that it may occur in the style only, with gametophytic control C, or independence G, in the pollen, in the pollen only, with embryosac control E, or independence H, in the style, or in both style and pollen J.

In all five systems only five genotypes are possible after one generation, as the homozygous top-dominant $\mathbf{S}1.1$ is never produced by compatible matings. The mating behaviour of the five genotypes is

shown in fig. 3. The dotted squares represent incompatible matings. The squares with the diagonal stroke in C and E are compatible cross-pollinations involving self-fertile seed parents which are, therefore, deemed unimportant and, for simplicity, ignored. In E however, only half the ovules of the three self-fertile genotypes are able to be self-fertilised. The other half are available to be shared among pollens with which they are compatible. These pollinations are represented by an incomplete diagonal enclosing the progeny genotypes. The half-occupied squares in C represent half-compatible matings. In calculating the various types of progeny of **S**2.2 and **S**3.3 as seed parents, therefore, account must be taken of the fact that only half the pollen of some parents is functioning and the contribution of these plants is weighted by $\frac{1}{2}$. The progenies obtainable from each relevant compatible pollination are shown within the square. All genotypes from one mating are equally frequent.

It will be noticed that H can be derived from G merely by interchanging the rows and columns. The pollinations of H behave as their reciprocals in G. The equilibrium frequencies are not identical, however, because of the effects of pollen competition.

The frequencies of all five genotypes in any generation can be expressed in terms of their frequencies in the previous generation. These frequencies are represented by the letters v to z. By equating the values of v to z for consecutive generations, equations relating to the equilibrium frequencies can be obtained.

In only one case, J, has a solution been obtainable algebraically and then only with the assistance of Dr J. A. Nelder, of the Vegetable Research Station, Wellesbourne.

In all five cases, as previously determined, there is a stable equilibrium with two alleles. Introduction of the third allele causes immediate unbalance, but the new equilibrium is achieved only after many generations.

Let the frequencies of	**S**1.2	**S**1.3	**S**2.2	**S**2.3	**S**3.3
be	v	w	x	y	z

C. At equilibrium, $v=x=0$; $z=1/3$ and $w+y=2/3$ both w and y having any positive value between 0 and 2/3. The third allele is in effect superfluous as there is no selective advantage in the persistence of either **S**1.2 (w) or **S**2.3 (y) provided one or other (they are both self-fertile) is available to pollinate the self-sterile **S**3.3. The position is essentially the same as when there are only two alleles (see table 1). It will be noticed that the only invariable as well as the commonest allele is **S**3, the bottom recessive, with a frequency of 2/3. If the population consisted in the first place of **S**1 and **S**2 only, at equilibrium $v=1/3$, $x=2/3$.

It can be shown that

$$v_{n+1}+x_{n+1}=v_n+\left(\frac{v_n+w_n}{v_n+2w_n+y_n+2z_n}\right)x_n$$

The above coefficient of x_n will be less than unity unless w, y and z are all zero. These three items all involve $\mathbf{S}_3$. Thus the introduction of the bottom recessive $\mathbf{S}_3$ to a population containing only $\mathbf{S}_1$ and $\mathbf{S}_2$ would lead eventually to the disappearance of $\mathbf{S}_{1.2}$ (v) and $\mathbf{S}_{2\cdot2}$ (x) with the establishment of the equilibrium given above. With the elimination of $\mathbf{S}_{2.2}$ the pollen of $\mathbf{S}_{3.3}$ ceases to have a function, encouraging male sterility just as with 2 alleles. On the other hand the introduction of either $\mathbf{S}_1$ or $\mathbf{S}_2$ into a population consisting of the other two alleles would have very little effect.

E. As an example in population genetics this is even more fluid than C. There is no single equilibrium frequency. If any arbitrary values are given to v, w, x, y and z there is a gradual change to an equilibrium peculiar to that set of initial values, the gene frequencies $\mathbf{S}_1$, $\mathbf{S}_2$ and $\mathbf{S}_3$ being close to their initial frequencies. If, therefore, a third allele is introduced to the system which previously had only two it will not tend to spread as in most other mating systems discussed herein.

G. It will be seen from fig. 3 that the five genotypes fall into three mating groups when considered as seed parents, but all behave differently as pollen parents. The only way in which I was able to determine the equilibrium frequencies was arithmetically, by choosing several arbitrary initial frequencies of the five genotypes and calculating the values in successive generations until they all converged on a stable set of frequencies, the equilibrium. The values thus obtained, correct to four decimals were:

$$\left.\begin{array}{l} v=0\cdot1217 \\ w=0\cdot2787 \\ x=0\cdot0202 \\ y=0\cdot3573 \\ z=0\cdot2221 \end{array}\right\} \text{ giving the gene frequencies } \left\{\begin{array}{l} \mathbf{S}_1=0\cdot2002 \\ \mathbf{S}_2=0\cdot2597 \\ \mathbf{S}_3=0\cdot5401 \end{array}\right.$$

H presents a very similar situation to G. The genotypes fall into five mating groups as females but three as males. The equilibrium frequencies (calculated in the same way) are slightly different from G:

$$\left.\begin{array}{l} v=0\cdot1722 \\ w=0\cdot2822 \\ x=0\cdot0359 \\ y=0\cdot3274 \\ z=0\cdot1823 \end{array}\right\} \text{ giving the gene frequencies } \left\{\begin{array}{l} \mathbf{S}_1=0\cdot2272 \\ \mathbf{S}_2=0\cdot2857 \\ \mathbf{S}_3=0\cdot4871 \end{array}\right.$$

J represents a simpler situation than G or H. The compatibilities as seen in fig. 3 are symmetrical. The five genotypes fall in to the same 3 mating groups whether as males or females. The three mating groups are intra-sterile and inter-fertile: $(v+w)$ forms group A, $(x+y)$ forms group B and z forms group C. It can be shown that an equilibrium is

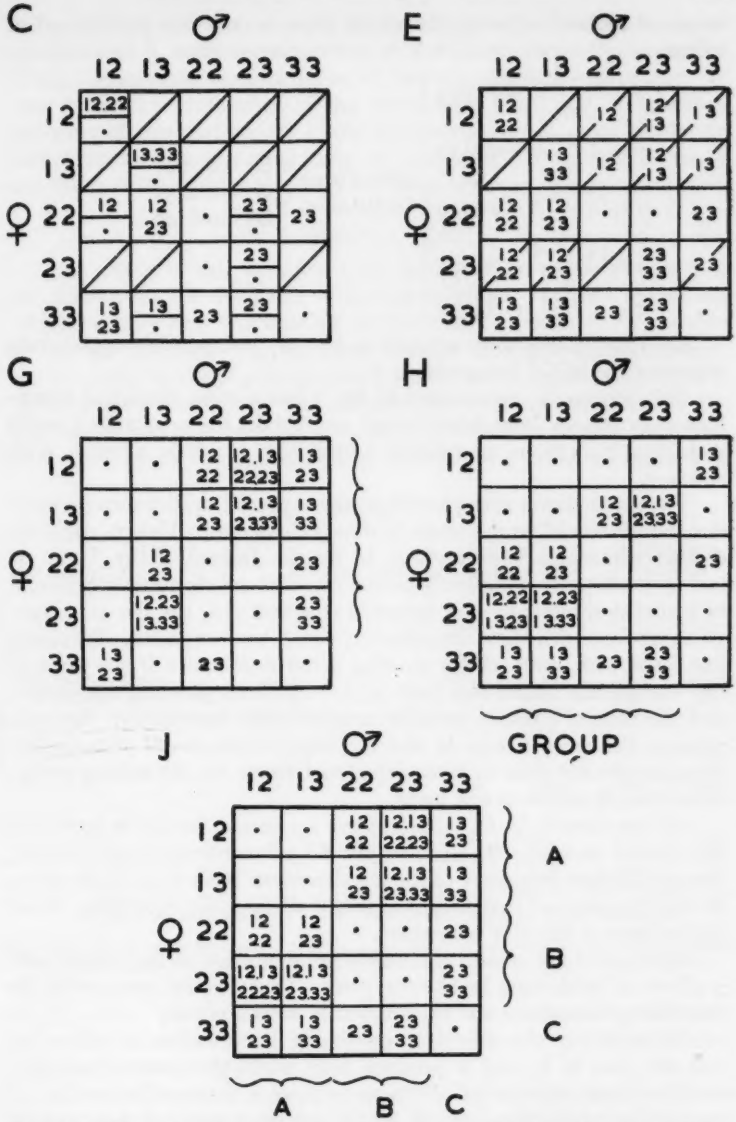


FIG. 3.—Possible matings in sample incompatibility systems with three alleles at one locus. There is sporophytic control accompanied by dominance on at least one side. When dominance occurs, the relationship is assumed to be $S_1 > S_2 > S_3$. The S-alleles are represented by their suffixes only. Incompatible pollinations have a dot in the square. Compatible cross-pollinations on to fully self-compatible seed parents have the square crossed through. In the remainder of the squares the progenies are represented. Where there is more than one genotype in a progeny, all are of equal frequencies. The cases (see table 1) are represented by the large letters. The half filled squares in (3) represent pollinations in which only half the pollen is compatible. The incomplete diagonals in (5) represent pollinations for which only half the ovules are available.

obtained if $A=B=C=1/3$ for which there is only one possible set of values:

$$\left.\begin{aligned} v&=\frac{2-\sqrt{3}}{3}=0{\cdot}0893\\ w&=\frac{\sqrt{3}-1}{3}=0{\cdot}2440\\ x&=\frac{7-4\sqrt{3}}{3}=0{\cdot}0239\\ y&=\frac{4\sqrt{3}-6}{3}=0{\cdot}3094\\ z&=\ 1/3\ =0{\cdot}3333 \end{aligned}\right\}\ \text{giving gene frequencies}\ \left\{\begin{aligned} \mathbf{S}1&=0{\cdot}1667\\ \mathbf{S}2&=0{\cdot}2233\\ \mathbf{S}3&=0{\cdot}6100 \end{aligned}\right.$$

For three alleles there appears to be only one possible equilibrium whatever the initial frequencies.

This scheme as represented in fig. 3 has a close empirical resemblance to Riley's hypothesis (1936) to explain the results in *Capsella grandiflora* though he postulated 2 loci with 2 alleles at each, with epistacy.

The resemblance extends even to the expected equilibrium frequencies, which would be the same as those calculated by Fisher (1949) for diploid trimorphic heterostyly as in *Oxalis*. Indeed, if the *S* and *M* loci of *Oxalis* were completely linked *MS* and *mS* chromosomes would be indistinguishable by any genetical tests and only the five genotypes given above (with those frequencies) could be recognised. Formally, one locus with three alleles showing serial dominance is the same as two completely linked loci each with two alleles showing dominance and the kind of epistacy peculiar to trimorphic heterostyly. But even without linkage between *M* and *S*, Fisher's estimates of *phenotype* frequencies are the same as those calculated above for the mating groups with three **S** alleles at one locus.

All five cases C, E, G, H and J have a common feature in favouring the bottom recessive $\mathbf{S}3$ and, where $\mathbf{S}1.2$ heterozygotes are formed, the equilibrium frequency of the top dominant $\mathbf{S}1$ is least of the three. It may be assumed that with a multiple allelic series recessivity would always have a selective advantage.

Space does not permit a detailed consideration of the effects with 3 alleles of weakening in heterozygotes. There would presumably be the same predominance of the self-fertile heterozygotes.

Summarising the effects of increasing the number of alleles we can say that B, D and F produce fully workable systems though F needs a large number of alleles to achieve a favourable amount of compatible pollination. G, H and J remain functional but without the peculiar features present with only two alleles. They become more recognisable as typical incompatibility mechanisms with a single equilibrium. The alleles are not, however, of equal frequencies at equilibrium. The more recessive a particular allele, the higher its equilibrium frequency. A, C and E are less suited to polyallelic systems.

All gene frequencies are stable in A. There is an infinite series of equilibria in E, each corresponding to a different initial frequency. In C the homozygous bottom recessive has an equilibrium frequency of one third, the remainder of the population consisting of arbitrary amounts of the various self-fertile heterozygotes. K, with self-fertile heterozygotes through weakening of competing alleles, will be much the same with additional alleles. L becomes even more improbable.

8. SEVERAL LOCI

The effects of this variation have already been partly discussed in the section on the evolution of incompatibility. To some extent the effects of increasing the number of alleles and increasing the number of loci are opposite. An increased number of alleles increases cross-compatibility in the population but has little effect on sib compatibility. An increased number of loci *decreases* the cross-compatibility in the population and also between sibs. As will be seen in table 2 the cross-compatibility in the population as a whole is generally higher than

TABLE 2

Incompatibility Control	No. of Alleles	Sib Cross-compatibility *a*		General cross-compatibility *b*		Outbreeding Efficiency *b/a*	
		1 locus	2 loci	1 locus	2 loci	1 locus	2 loci
♂Gametophytic: ♀Sporophytic	3	0·25	0·063	0·33	0·11	1·33	1·78
with independence	4	0·30	0·09	0·50	0·25	1·60	2·76
Case B	∞	0·50	0·25	1·00	1·00	2·00	4·00
♂ and ♀ Sporophytic	4	0·25	0·063	0·167	0·028	0·67	0·44
with independence	5	0·25	0·063	0·300	0·940	1·20	1·44
Case F	∞	0·25	0·063	1·000	1·000	4·00	16·00
♂ and ♀ Sporophytic	2	0·50	0·25	0·50	0·25	1·00	1·00
with dominance	3	0·52*	0·27	0·67	0·44	1·29	1·67
Case J	∞	*ca.* 0·50	*ca.* 0·25	1·00	1·00	*ca.* 2·00	*ca.* 4·00

* This value obtained from Fisher's calculations (1949) for *Oxalis valdiviensis*

The effects of increasing the number of alleles and number of loci on cross-compatibility between sibs and amongst the general population from three types of incompatibility system.

among sibs. This ratio can be regarded as the outbreeding efficiency of a particular incompatibility system (the absence of self-fertilisation being a constant feature).

It must be stated here that since the incompatibility reaction is between pollen and style the degree of compatibility between plants should be expressed in terms of compatible pollen. With sporophytic control this is the same as the proportion of compatible plants. With gametophytic control of pollen such is not the case. Thus, both $\mathbf{S}_1\mathbf{S}_3$ and $\mathbf{S}_3\mathbf{S}_4$ plants are compatible as pollen parents with $\mathbf{S}_1\mathbf{S}_2$ but under open pollination (with pollen competition) pollen of $\mathbf{S}_1\mathbf{S}_3$ will

only be half as likely to effect fertilisation as pollen of $\mathbf{S}_3\mathbf{S}_4$. Hence, in table 2, the cross-compatibility between sibs when there is gametophytic control and a large number of **S**-alleles is 0·5 and *not* 0·75 as stated by Fisher (1949). (Parent-offspring compatibility is also 0·5 and *not* 1·0.) This gives such a system a higher outbreeding efficiency than trimorphic heterostyly whether in respect of sib mating or parent-offspring mating. As shown earlier, trimorphic heterostyly resembles complete sporophytic control with three alleles at one locus showing dominance, which is entered in table 2.

An increase in number of loci from one to two, squares the frequencies of cross-compatibility. It thus squares the ratio of general to sib compatibility. Where, therefore, the outbreeding efficiency with one locus is greater than unity (as is usual) it is increased with two loci. When it is less than unity, in the special case of independent sporophytic control with one locus and four alleles, it is smaller still with two loci. With one locus and two alleles with dominance (as in dimorphic heterostyly), the outbreeding efficiency is unity. It remains so with two loci. Even when we consider only those instances in which increase in loci raises the outbreeding efficiency it is doubtful whether this effect would compensate the species for the general reduction of cross-compatibility, especially since, as argued earlier, the effect upon sib mating is unlikely to have a high selective value.

It is conceivable, however, that two loci could show interaction in the form of epistacy such that only one locus at a time was operating. The cross-compatibility could then remain high. Such a situation was proposed by Riley (1936) to explain his *Capsella grandiflora* data. The two loci were T, t and S, s with T epistatic to S.

9. SUMMARY

1. The possible range of self-incompatibility systems is derived *a priori*, and known systems are related to it.

2. These may act either by *promoting* the growth of pollen from genotypes *unlike* that of the style, or by *inhibiting* the growth of pollen from genotypes *like* that of the style. These are called complementary and oppositional mechanisms. Possible examples of both types are given. Oppositional mechanisms give the greatest evolutionary scope and are the most widespread.

3. Other classifications of incompatibility: hetero-*v.*, homomorphic, di- and tri-*v.* multipolar and haplo-diploid *v.* diplo-diploid are compared analytically.

4. Incompatibility has probably arisen *de novo* many times. Consequently all workable incompatibility systems are likely to be encountered in nature, and in all stages of development.

5. Self-incompatibility would probably evolve gradually, possibly from a condition of several weakly acting loci, by selection of modifiers to a condition where one or few main loci were active.

6. The workings of sporophytic control with independence, dominance or weakening in the interaction between alleles, of gametophytic control, and of varying numbers of alleles at one or more loci are discussed separately and in many combinations.

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Notes added in proof:

[1] Since writing the above Dr D. Lewis has published a paper (1952, Serological reactions of pollen incompatibility substances. *P.R.S.*, B, *140*, 127-135), showing a se rological resemblance between pollens with common **S**-alleles, bringing one stage ne arer the demonstration that the incompatibility reaction does involve antigens.

[2] In a private communication Dr Å. Gustafsson claims to have found self-incompatibility in *Pinus sylvestris*, thus proving that the absence of a style does not prevent gymnosperms from evolving self-incompatibility.

[3] Recent unpublished work by D. Wilkie of Glasgow has found in the pteridophyte *Pteridium aquilinum* a wholly gametophytic self-incompatibility quite analogous to case A, with a selective value much the same as in angiosperms. Thus the improbability of finding an example of case A in angiosperms is reduced.

POLYGENIC INHERITANCE OF DRUG-RESISTANCE IN THE BACTERIUM *ESCHERICHIA COLI*

L. L. CAVALLI AND G. A. MACCACARO
Department of Genetics, University, Cambridge

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1. INTRODUCTION

THE inheritance of quantitative characters has been open to statistical investigation in higher organisms since Fisher's paper (1918) on the correlation between relatives. The evidence thus accumulated (Mather, 1949; Lerner, 1950) has favoured the view early announced (Nilsson-Ehle, 1909; East, 1915) that the inheritance of such traits follows the rules of Mendelian inheritance, assuming that many genes contribute to the total effect. The importance of polygenic systems for higher organisms is to-day documented well enough not to need further emphasis.

An investigation of selection for drug resistance in bacteria led Demerec to postulate the existence of such polygenic systems whenever a high level of resistance could be attained only by a number of repeated selections, each determining a moderate increase of the resistance level. Demerec called " first-step " resistants, organisms isolated after a single exposure to the drug. Penicillin resistant first-steps isolated from sensitive strains of *Staphylococcus aureus* have as a rule acquired a minor degree of resistance and higher levels are attainable only stepwise. Streptomycin-resistant first-steps, on the other hand, showed a wide range of variation of the resistance level thus acquired, including full resistance. Contrasting these two types of behaviour, Demerec proposed for them the names of " penicillin " and " streptomycin-pattern " respectively.

The absence of a known sexual phase in *Staphylococcus aureus* and the other organisms in which penicillin resistance can be investigated prevents a test of the polygenic hypothesis. On the other hand, the discovery of recombination in *Escherichia coli*, strain K-12, by Lederberg and Tatum (1946) offered the basis for Mendelian analysis in at least one bacterial strain.

Streptomycin resistance in K-12 was thus investigated by Newcombe and Nyholm (1950*a*), by Demerec and by others and all resistant or dependent mutants tested proved to be allelic or due to very closely linked loci. No other cases of drug resistance showing the streptomycin-pattern were described, unless one considers (somewhat loosely in respect of the original definition by Demerec) resistance to sodium azide, introduced as a chromosome marker by Lederberg, to belong to the streptomycin-pattern. Azide resistance also is apparently due to a single locus (according to data mostly unpublished by various authors).

On the other hand, the " penicillin-pattern " seems a common mode of behaviour towards many drugs. It is clear enough that whenever selection leads to a gradual increase in resistance, if the mechanism is genic, polygenes must be at work. The first case that offered itself to observation in *E. coli* K-12 was resistance to nitrogen-mustard (Cavalli, unpublished).

Nitrogen-mustard resistance increased more or less continuously in successive transfers in broth tubes with increasing amounts of the drug; inheritance in crosses suggested more than one locus being involved but the extreme instability of the drug made a detailed study far too difficult.

Chloromycetin (chloramphenicol; henceforth abbreviated as Chl) was the next drug tested in *E. coli* K-12 which showed a very definite " penicillin-pattern ".

On first exposure to the drug on Chl-agar only a moderate increase can be attained, from 2 up to (seldom) 5 times the initially tolerated level (approx. 5γ/ml.). On the other hand, selection by the common bacteriological procedure known as " training ", i.e. successive transfers in increasing concentrations of the drug, gave increases of resistance up to more than 1000γ/ml., that is more than 200 times the initial level. Resistance seemed to increase very gradually, roughly twofold at every transfer, and thus the case seemed to provide excellent material for testing the hypothesis put forward by Demerec, namely that resistance of the " penicillin-pattern " is due to a polygenic system with no major genes involved.

2. MATERIAL AND METHODS

E. coli K-12 strains employed in this investigation were all derivatives of 58-161, which is a biotinless-methionineless strain* and Y-10 which is a threonineless, leucineless, Vitamin B_1-less strain.

TABLE 1

Phenotypes of strains used in crosses (all from E. coli *K-12)*

Own designation	Lederberg's designation (or origin)	Biochemical deficiencies (B) M T L B_1	Sugar fermentations Lac Gal Mal Xyl	Virus resistances V1 V6	Drug resistances Az St
2	58-161	− − + + +	+ + + +	s s	s s
26	W-705	− − + + +	− − − +	r s	s s
30	W-583	+ + − − −	− − − −	r s	s s
36	Y-10	+ + − − −	+ + + +	s s	s s
47	W-677	+ + − − −	− − − −	r s	s s
98	from Y-10	+ + − − −	+ + + +	s r	s s
100	from 58-161	− − + + +	+ + + +	s r	r s
102	from W-677	+ + − − −	− − − −	r r	r s
123	from Y-10	+ + − − −	+ + + +	s s	s r

* Although 58-161 was stated to be biotinless, this deficiency was actually either lost or undetectable in the strain in our hands, since 58-161 grows well on minimal supplemented with dl-methionine alone. Absence of biotin from constituents of the medium was tested by means of *Lactobacillus casei* assay. However the methionine requirement is an excellent marker, even used singly, as back-mutants are exceedingly rare on control plates.

These were the markers used for recombination, which was nearly always carried out by inoculating 10^8-10^9 cells of each strain on the surface of minimal agar plates, prepared with the medium suggested by Lederberg, and occasionally supplemented with Vitamin B_1.

Table 1 gives the phenotypes of 58-161, Y-10 and some derivatives of them kindly sent by J. Lederberg or prepared in this laboratory, which were employed throughout these experiments (markers not used were excluded).

Prototrophs, *i.e.* colonies growing on the minimal medium, were scored for segregating markers according to the methods given by Lederberg (1947). In most crosses however, in order to avoid parental contamination, which might have been of nuisance for resistance testing, each prototrophic colony was plated on complete agar, one colony picked at random and tested for prototrophism while scored for markers. Non-prototrophic colonies isolated in these conditions were exceptional. Care was taken to avoid unconscious selection due to colony size and shape by this procedure, as well as when picking prototrophs from the plates where crosses had taken place.

The following linear order, as after Lederberg (1947) with minor additions was found to be a satisfactory guide to interpret the results of the crosses:

$$B_1 - (B)\ M - Gal - V_6 - Lac\ V_1 - Az - (TL)$$

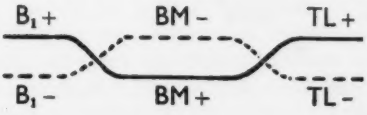
while Xyl, Mal, Ara, were found to be linked in a complex, unexplained way with M (see for instance Newcombe and Nyholm, 1950; Cavalli, 1950).

Gal refers to the Gal locus of strain W583. Such a mutation is not allelic with the Gal factors of W677 and W705, which are linked with Xyl, Mal, Ara.

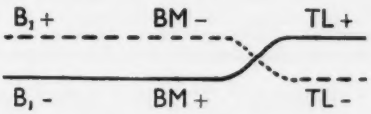
Although the interpretation of the results indicated below is by no means dependent on the above hypothesis, this last was found to be a useful aid, in wait of an improved understanding of the situation.

Therefore the patterns of selection of recombinants were the following:

(i) when selecting full prototrophs, *i.e.* on minimal agar:



while (ii) when selecting on agar supplemented with Vitamin B_1, the majority (90 per cent) of the recombinants are obtained by exchange of the type:



where solid lines indicate the recombinants selected for, and dotted lines indicate the hypothetical multiply deficient recombinants.

Resistance tests were carried out, except when otherwise stated, by streaking on agar plates containing various concentrations of Chloromycetin.*

At the beginning, a 2-fold increase in concentration was used starting froms 1.25 γ/ml.; in later experiments a scale of concentrations with a logarithmic increase of 1/9 was introduced starting from 1 γ/ml. or higher, as it was found that the streaking

* Chloromycetin can be added and sterilised in autoclave without appreciable reduction of activity, which makes the preparation of Chloromycetin agar straightforward. Chloromycetin, Parke and Davis, was especially but not exclusively employed.

technique could bear such a refinement, provided the cell concentration of suspensions and amounts streaked were standardised in a satisfactory way.

Parallel streaks of different resistant and sensitive organisms did not show any interaction due to Chl destruction or similar phenomena.

Moreover, although Chl can be destroyed by bacteria, as is well known (Smith and Worrell, and others), out of three highly resistant strains only one showed an increased capacity to destroy Chl, as compared with the sensitive strains (Magistretti, unpublished). Destruction can be achieved only by very high concentrations of bacteria.

Growth of streaks on Chl agar was scored after 24 and/or 48 hours. A very high correlation between the observations at the two times was found. However, scoring at 48 hours was preferred, since growth in presence of the drug is delayed.

When more accurate tests of resistance were wanted, suitably diluted suspensions of the strains to be tested were plated on agar with various concentrations of Chl, and in controls without the drug and colony counts made after 3 days of incubation.

The symbolism adopted for Chl-resistant strains is Chl^r; when a number precedes the letter *r* in the superscript it refers to the highest tolerated level, when it follows *r* it denotes the number of the resistant strain (*e.g.* Chl^{320r}, *i.e.* a strain resistant to 320γ/ml., and Chl^{r11}, *i.e.* resistant strain No. 11). The normal level of Chl-sensitivity can be indicated by Chl^s, but this symbol is usually omitted.

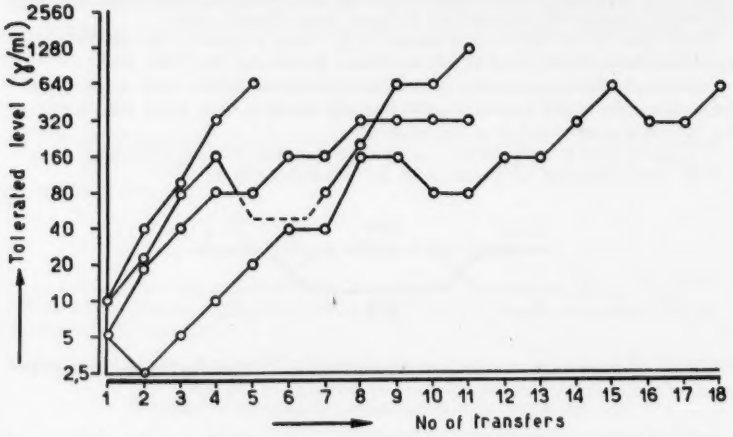


FIG. 1.—"Training" to high resistance to chloroamphenicol (Chl) by successive transfers in a series of concentrations of the drug in broth. Abscissae: number of transfers; ordinates: maximum tolerated concentration in γ/ml. Four independent experiments.

3. "TRAINING" TO HIGH RESISTANCE

K-12 strains can grow in broth containing about 5γ/ml. of Chl, often with some delay. With heavy inocula growth can occur even in concentrations as high as 20γ/ml. Subculturing on to higher and higher concentrations "training" to higher concentrations is regularly observed.

Fig. 1 shows increases in resistance such as were observed in four such independent experiments. As growth is usually delayed, especially with high concentrations, cultures were incubated 48 hours before every transfer (·05 ml. in 10 ml.).

When the highest tolerance levels are reached growth becomes scanty with, as without, the drug. As can be seen from the curves in fig. 1 the progress towards high resistance is often smooth, although regression to lower resistance can be observed. No clear-cut steps are recognisable. The highest levels thus reached were not far from the water solubility limit of Chl, about 2 per cent according to our experience. When highly resistant strains were transferred on Chl-free media their resistance decreased from 320γ/ml. to 80γ/ml. in one month of frequent subcultures, while if subcultures were made less frequently (once weekly or less) resistance kept to the high level, or nearly so, during the same time.

Reversion is not surprising, since highly resistant strains are impaired in their growth capacity in normal media and therefore back-mutants should be favoured. The maintenance of cultures at their original resistance level for long periods seemed in fact impossible, even if cultures were kept in the refrigerator and subcultured at three months intervals. Many cultures seemed to stabilise at an intermediate level of resistance. No Chl-dependent organisms were found, although they were especially looked for by the penicillin screening technique.

Merkel Hermann and Steers, working with *E. coli B*, carried out selection for resistance to Chl in minimal media and in broth and found that in the latter case resistance was acquired more rapidly and was accompanied by several requirements for aminoacids. In strains selected by us no absolute requirements for extra growth-factors, additional to those already necessary for growth of the normal sensitive strains, were found; although growth in minimal medium, supplemented by the necessary growth-factors, was delayed in respect of controls. No appreciable alterations of other biochemical characters were found, but this experience was limited to the markers in common use for this work. The only important exception to this rule was a marked tendency of most resistant strains to give mucoid colonies on carbohydrate rich media.

Three highly resistant strains: one out of #47 (#47 Chl^{640r}) and two out of #2 (#2 Chl^{320r} and #2 Chl^{1280r}) were crossed with suitable sensitive strains (#2 and #47 respectively). Prototrophs obtained on minimal medium were tested for Lactose fermentation, V_1 resistance and resistance to Chl by streaking on agar plates containing Chl from 10γ/ml. to 320γ/ml. or more in twofold increasing concentrations.

The distributions of the numbers of prototrophs obtained in this way are shown in table 2.

Now it is clear that if high resistance is due to a number of genes scattered along the chromosome and interacting additively, the prototrophs arising by recombination from a sensitive and a highly resistant strain should be intermediate in resistance between the parents but with a very variable degree of resistance, which should be roughly proportional for individual prototrophs to the length of the segment which the given prototroph has obtained from the resistant parent. This is

TABLE 2

Distribution of prototrophs according to Lac V_1 phenotype and Chl-resistance in crosses: highly resistant × sensitive strains

Lac V_1	<10	10	20	40	80	160	320	Totals	Geometric means of resistance
(I) Cross: #2 × #47 Chl^{320r}									
	Chl-resistance (γ/ml.)								
+ s	17	6	21	4	2	1	—	**51**	12
− s	1	1	7	12	3	2	—	**26**	35
− r	—	2	8	10	10	6	—	**36**	49
+ r	1	2	1	—	—	1	—	**5**	18
Totals	**19**	**11**	**37**	**26**	**15**	**10**	—	**118**	25
(II) Cross: 2 #Chl^{1280r} × #47									
Lac V_1	<10	10	20	40	80	160	320	Totals	
+ s	10	20	2	13	5	—	—	**50**	18
− s	49	74	3	2	2	1	—	**131**	8·4
− r	43	30	3	2	1	—	—	**79**	7·5
+ r	2	5	1	3	—	—	—	**11**	14
Totals	**104**	**129**	**9**	**20**	**8**	**1**	—	**271**	9·3
(III) Cross: #2 Chl^{320r} × #47									
Lac V_1	<10	10	20	40	80	160	320	Totals	
+ s	8	13	13	12	3	—	—	**49**	17
− s	75	16	4	2	1	—	—	**98**	6·4
− r	31	16	3	—	1	—	—	**51**	7·1
+ r	1	—	—	—	—	—	—	**1**	5
Totals	**115**	**45**	**20**	**14**	**5**	—	—	**199**	8·4

borne out by the experiments. In fact the average resistance (measured as geometric mean) for the class of recombinants Lac + V_1^s in cross 1 of table 2 is less than that of recombinants Lac − V_1^s, which, having crossed over in the second segment, take a greater proportion of their chromosome from the Chl^rLac − V_1^r parent. Lac − V_1^s recombinants are in turn less resistant than Lac − V_1^r (although this latter difference is not significant). In the reverse crosses, where the Lac + V_1^s parent is the resistant

TABLE 3

Crosses between highly resistant strains

Lac	V_1	<10	10	20	40	80	160	320	640	Totals
(I) Cross: #2 Chl^{320r} × #47 Chl^{320r}										
+	s	—	—	20	18	6	1	—	—	**45**
−	s	3	—	19	42	5	5	1	—	**75**
−	r	15	—	6	45	3	3	—	—	**70**
+	r	—	—	3	6	—	—	—	—	**9**
Totals		**16**	—	**48**	**111**	**14**	**9**	**1**	—	**198**
(II) Cross: #2 Chl^{1280r} × #47 Chl^{320r}										
Lac	V_1	<10	10	20	40	80	160	320	640	Totals
+	s	11	3	7	12	12	2	—	—	**47**
−	s	4	6	11	18	26	10	1	—	**76**
−	r	5	4	3	6	9	1	—	—	**28**
+	r	—	—	2	1	2	—	—	—	**5**
Totals		**20**	**13**	**23**	**37**	**49**	**13**	**1**	—	**156**

one, Lac $-V_1^s$ are more resistant than Lac $+V_1^s$. It should be noted that the average resistance of all recombinants is always significantly smaller than the (geometric) mean resistance of the parents. The geometric scale was chosen for all comparisons because of the nearly geometric increase of resistance during selection, especially in its early phases. We shall see later that this diminution of resistance in the prototrophs is well compatible with a polygenic system, in which non-additive interactions occur.

When highly resistant strains were crossed between themselves the distributions of prototrophs given in table 3 were found. These results are again contradictory of a simple polygenic hypothesis in which all polygenes react additively together. In fact they show that fully sensitive prototrophs can appear. This cannot be due simply to the condition that the genes in the resistant strains crossed together are all non-allelic. This would not explain why no prototroph with resistance higher than that of either parent was recorded. Also it would not explain why such fully sensitive prototrophs are, as they often are, bad growers. Rather, it is likely that either (*a*) negative interaction can occur between two genes for resistance, perhaps affecting growth rate as well; (*b*) that some genes may act as " modifiers " of the resistance due to other genes, determining an increase of it, without having any effect on resistance in the absence of the genes the expressivity of which they modify.

Such hypotheses were tested by crossing three sensitive prototrophs (arising from the cross between the two highly resistant strains mentioned above) to #123, which is a TLB_1 requiring, streptomycin resistant, Chl sensitive strain, on minimal medium supplemented with streptomycin. The Lac V_1 phenotype of the three sensitive prototrophs was -s, -r, -r respectively and all of them did not grow on 10γ/ml. of Chl. There is no cross resistance between Chl and streptomycin. The two latter prototrophs (both -r) crossed to $TLB_1 - St^r$ on minimal agar supplemented with streptomycin gave all Chl sensitive prototrophs. The -s prototroph crossed in similar conditions to the $TLB_1 - St^r$ strain gave (out of 48 recombinants):

3 Chl sensitives
43 resistants to 16γ/ml of Chl
2 resistants to 25γ/ml of Chl

However, when this cross was later repeated, using strains derived from 3 colonies isolated from the -s prototroph, one of which was a good grower and the other two poor growers, no recombinants with a resistance clearly higher than that of either parent was recorded. The problem was not analysed further on these data. The whole question of interaction between genes will be discussed later.

4. ANALYSIS OF SINGLE STEPS

The process of " training ", *i.e.* in the present case the attainment of resistance by a number of successive transfers in liquid media con-

taining higher and higher concentrations of the drug, gave rise, for Chl, to results which are best interpreted on the basis of a polygenic system. If it is possible to obtain resistant individuals, in which one single gene-difference is responsible for resistance, they should give, on crossing to sensitives, clear-cut segregations into resistants—all showing the same level of resistance—and full sensitives, in marked contrast with the results obtained with strains derived by means of long selection, *i.e.* " training ".

Such a single-step-resistant should be obtainable either by (i) stopping the training process after the first exposure to the drug, or (ii) by plate selection procedures such as were used by Demerec in his first investigations, and by others.

By the latter method a large number of cells (of the order of 10^9 cells) of the sensitive strain, from cultures originated from a small inoculum, were plated on Chl-agar. Provided the concentration of the drug is between 20 and 40 γ/ml. a few colonies will develop after the first day of incubation and many more on the second and third day. As shown elsewhere (Cavalli, 1952) colonies developing later are on average less resistant than those developed earlier. Fluctuation tests showed a high variability of the number of resistant colonies derived from parallel independent sensitive cultures; but a closer analysis according to Luria and Delbrück's model, developed by Lea and Coulson, is prevented by the difficulty of obtaining reliable counts of resistant colonies from the same culture. Such difficulties are in part due to the extreme variability of size of the resistant colonies, many of which are near the threshold of visibility.

The great variability of size should be a consequence of the great variability of the time of onset of growth of individual resistants, and this is, partly at least, caused by variability of the resistance level of independent single steps.

Resistants which were presumably single steps were isolated by both techniques and crossed to sensitives.

Table 4 shows the results of such crosses for two resistants isolated after one transfer of #26 in Chl-broth (20 γ/ml.) under shaking for 48 hours, showing a different degree of resistance. If a line is drawn dividing individuals growing on 5 γ/ml. but not on 10 γ/ml. and those growing on 10 γ/ml. and more, considering the former as sensitives, the latter as resistants, one can map a locus for Chl-resistance between M and V_6. One thing worth noting is that the first resistant Chl^{20r} which reaches the resistance level of only 20 γ/ml., gives a segregation which is superimposable on that obtained with the second resistant Chl^{40r} which did, instead, grow on 40 γ/ml. In either cross the resistant prototrophs show resistance to 10 or 20 γ/ml., never higher, which is approximately the same as that shown by the first resistant parent Chl^{20r} (especially considering that the two normal strains #26 and #98 are not identical in resistance to Chl, the former being spontaneously somewhat more resistant; see later). Since the two resistants

are not independent in origin, because they were isolated out of the same selection tube, *i.e.* Chl-broth, the easiest explanation is that in the condition employed for selection in liquid medium, even after one exposure a second step of resistance can be reached. Growth under shaking, as here, gives in fact excellent conditions for rapid selection and it is not impossible that in one cycle of growth in liquid medium

TABLE 4

Crosses between sensitive strains and "first step" resistants, obtained by selecting for resistance in liquid media

Strains:	#26	M −	V_6^s	Lac −	V_1^r	TL +
			a	b	c	d
	#98	M +	V_6^r	Lac +	V_1^s	TL −
(Cross I): #26 Chl[20r] × #98						
V_6Lac V_1phenotype exchange in region	s − r a	r − r b	r + r c	r + s d	s + r abc	s + s abd	s − s acd	r − s bcd
Chl-resistance < 5 γ/ml.	3	10	66	64	—	2	1	1
5 γ/ml.	4	1	6	9	1	3	—	—
10 γ/ml.	20	—	1	1	—	—	2	—
20 γ/ml.	1	—	—	—	—	—	—	—
40 γ/ml.	—	—	—	—	—	—	—	—
Totals	**28**	**11**	**73**	**74**	**1**	**5**	**3**	**1**
(Cross II): #26 Chl[40r] × #98								
	s − r	r − r	r + r	r + s	s + r	s + s	s − s	r − s
< 5 γ/ml.	1	6	73	59	—	1	—	1
5 γ/ml.	8	3	6	7	1	—	—	1
10 γ/ml.	11	1	1	1	—	—	4	—
20 γ/ml.	4	1	—	—	—	—	—	—
40 γ/ml.	—	—	—	—	—	—	—	—
Totals	**24**	**11**	**80**	**67**	**1**	**1**	**4**	**2**

more than one step of resistance can be climbed. According to this interpretation the first resistant strain, Chl[20r], was the first step and the second resistant Chl[40r] had gone one step further, the second step gene being closely linked with M and therefore not showing among segregants.

When first steps obtained by plate selection were analysed with crossings, results interpetable in terms of one only gene-difference were the rule, except in a case which will be discussed later.

Out of ten Chl[r] genes that were mapped in crosses of first or second steps with sensitives, two independent ones were closely linked with M; one was linked with M but more loosely, showing some linkage with an "unmappable" marker, Gal; three were between M and V_6; one between V_6 and Lac and one probably right of Lac; one was between V_1 and TL and one (to be analysed later) was unlinked with genes between M and TL.

Thus there is not a fixed locus, mutation at which is responsible for

first step resistance, but rather a number of loci each of which can determine this effect.

The above data in which resistance was tested by the streaking technique can give satisfactory results as far as mapping is concerned, but will not answer the question whether resistance of single steps is actually segregating in a discrete way, as is to be expected for a mechanism of particulate inheritance, in contrast with the expectation of blending inheritance. To this aim more refined tests for resistance

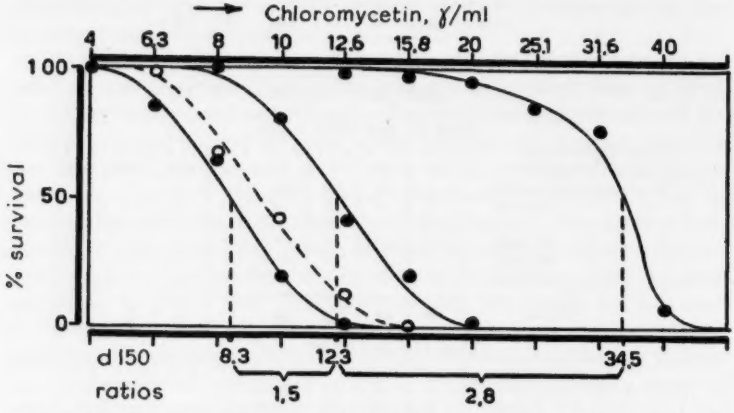


FIG. 2.—Resistance curves of four strains. Abscissae, concentration of Chl; ordinates, percentage of survival. The four curves, from left to right, belong to: #100, sensitive; #47, sensitive (dotted line); #100 Chl^{r8}, first-step resistant from #100; #100 $Chl^{r8/r9}$, second-step resistant from same.

are necessary. The following was used in the present case. Resistant and sensitive strains and prototrophs from them were tested by plating a suitable number of cells (of the order 100-300) in agar plates, and in agar containing various concentrations of Chl. After three days of incubation the number of colonies developed was counted and survival "percentages" calculated as the ratios of the number of colonies on agar with a given amount of Chl/ml. and the number of colonies on drug-free agar plates.*

Survival curves obtained for two parental sensitive strains (#100 and #47) are given in fig. 2 and it can be noted that these strains show some difference of sensitivity. This slight increase of resistance seems to be often but not always associated with the Lac – V_1^r phenotype and was the cause of some trouble in interpretation of segregations (see also before, *re* experiments reported in table 4).

In fig. 2 are also shown the survival curves of the first step resistant

* These experiments were carried out in another laboratory (Istituto Sieroterapico Milanesi, Milan) and with other media, so that it is not surprising that the resistance levels have shifted from those previously reported.

Chl^{r8} derived from #100 by plate selection and of the second step resistant Chl$^{r8/r9}$ derived from Chl^{r8} by a second plate selection. The increase in resistance observed in Chl^{r8} is exceedingly slight, the 50 per cent tolerated dose having increased only 1·5 times. In spite of this a segregation of resistance is recognisable among recombinants from #100 Chl^{r8} × #47 in the form of a bimodal distribution, as can be

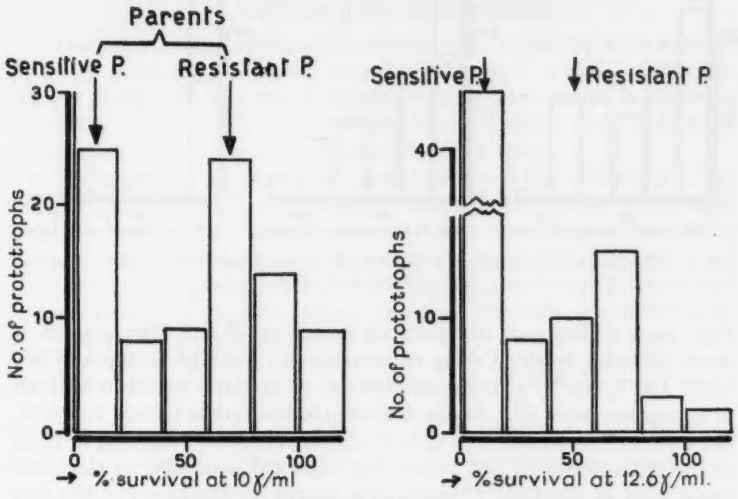


FIG. 3.—Distribution of resistance of prototrophs from cross #100 Chl^{r8} × #47. Resistance is indicated on the abscissae as " percentage " of survival at a given drug concentration. In the left hand figure, resistance at 10 γ/ml.; in the right hand figure at 12·6 γ/ml.

seen from figs. 3*a* and 3*b*, representing the distribution of resistance of the prototrophs from cross #100 Chl^{r8} × #47, where the scale in which resistance is measured is survival percentage at a given concentration of the drug. Two drug levels are indicated (10 and 12·6 γ/ml.). In the histograms the arrows indicate the resistance levels of the two parents and it can be seen that they correspond approximately with the two modes. It is not surprising that the distribution of resistance of the prototrophs, although being bimodal, is not clearly discontinuous. The difference in resistance between the parents is small, in comparison with the standard deviation due to error in the measure of the individual resistance (about ± 10 per cent). Also the existence of some difference in resistance between #100 (the normal parent of Chl^{r8}) and #47 will undoubtedly contribute to obscure the results.

If an arbitrary distinction is made at a survival of say 20 per cent, in fig. 3*a*, to classify sensitives and resistants, no linkage between resistance and the usual Lac V_1 markers is found (data not shown). This means, as far as present linkage tests can say, that the gene for resistance in strain Chl^{r8} is outside the marked region. When the second step

Chl$^{r8/r9}$ is crossed to sensitive #47 and the distribution of resistance of prototrophs is analysed in the same way (fig. 4) a more complex distribution is expected and indeed found.

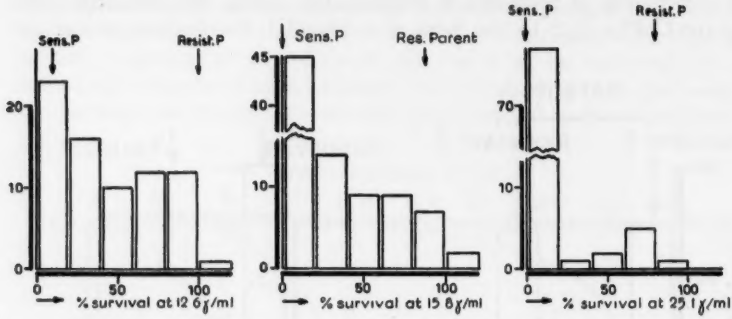


FIG. 4.—Distribution of resistance of prototrophs from cross #100 Chl$^{r8/r9}$ × #47. Abscissae and ordinates as in fig. 3.

Figs. 4*a*, *b*, *c* show such distributions as they result from the analysis at three different levels of drug concentration. Four phenotypes (Chl^{s}, Chl^{r8}, Chl^{r9}, Chl$^{r8/r9}$) are expected in this cross, but it would be difficult to recognise them all. At the highest concentration tested, however, the action of the second step gene is clearly recognisable, giving a mode at the resistance level just below that expected according to the resistance level of the Chl$^{r8/r9}$ parent (as shown by the arrow). We can assume—somewhat arbitrarily of course—that 20 per cent survival at 25 γ/ml. represents the resistance level which divides recombinants of this cross into those possessing the second step gene Chl^{r9} (irrespectively

TABLE 5

Cross with #100 Chl^{s} of second step resistant #100 Chl$^{r8/r9}$

Chl-resistance scored according to survival at 25 γ/ml.; Chl^{r}, prototrophs with survival percentage >20 per cent, Chl^{s} at survival percentage less than 20 per cent.

Markers region	M	Gal	V_6	Lac	V_1	Az	TL
		a	b	c	d	e	f

Phenotype Gal	V_6	Lac	V_1	Az	Exchange region	No. of prototrophs Chl^{s}	Chl^{r}
+	r	+	s	r	a	(1)*	3
−	r	+	s	r	b	0	1
−	s	+	s	r	c	11	5
−	s	−	s	r	d	40	0
−	s	−	r	r	e	14	0
−	s	−	r	s	f	7	0
various combin. (triples)					abd, ade aef; $c_1$de, adf	3	2
					Totals	76	11

* uncertain classification for resistance

of whether they also possess the first step Chl^{r8}) and those not possessing it. If we score prototrophs Chl^{r9} and Chl according to this assumption, and examine their distribution according to Chl^{s} and the other markers analysed in this cross (Gal V_6 Lac V_1) we find that Chl^{r8} must be between V_6 and Lac (see table 5). The data are scanty because of the tediousness of the procedure of determining resistance.

5. ALLELISM AND INTERACTION

From the previously reported experiments a relatively large number of genes seems responsible for behaviour in respect of Chl-resistance in *E. coli* K-12. Only a few preliminary tests were made in order to ascertain (i) how often are two independent mutants allelic, (ii) what interaction can one expect between non-allelic genes.

The following experiment is a contribution to the problem (i): Ten independent first steps were selected from #2 by plating fully grown cultures started from about 100 cells each on Chl-agar containing 25 γ/ml. Similarly seven such first steps were selected from #47: one first step from #47 was crossed with each of ten first steps from #2 and the proportion of sensitive prototrophs thus obtained was determined.

The results are given in table 6.

TABLE 6

Allelism tests of first steps

Percentages of Chl-sensitives and number of prototrophs tested in 15 crosses

Strains derived from: #100	#102	% Chl^{s}	No. of prototrophs
Chl^{r11} ×	Chl^{r21}	21·1	52
Chl^{r12} ×	,,	6·2	112
Chl^{r13} ×	,,	0·9	109
Chl^{r14} ×	,,	24·7	81
Chl^{r15} ×	,,	1·3	75
Chl^{r16} ×	,,	0·8	252
Chl^{r17} ×	,,	5·4	112
Chl^{r18} ×	,,	0·0	85
Chl^{r19} ×	,,	1·8	109
Chl^{r20} ×	,,	3·2	95
Chl^{r17} ×	Chl^{r22}	82·3	17
,, ×	Chl^{r23}	56·0	25
,, ×	Chl^{r26}	25·2	111
,, ×	Chl^{r27}	16·1	112

Only in one out of the 15 crosses were all prototrophs resistant and perhaps even in this case—had more recombinants been investigated—a few sensitives might have arisen. Moreover there is a significant difference between the proportions of sensitives obtained in the various crosses.

At least four or five different loci should be invoked in order to explain the results: one locus for Chl^{r21} and Chl^{r18}, one locus for Chl^{r12}, Chl^{r13}, Chl^{r15}, Chl^{r16}, Chl^{r17}, Chl^{r19}, Chl^{r20}, one for Chl^{r11} and Chl^{r14}, one for Chl^{r22} and Chl^{r23}, one, perhaps, for Chl^{r26} and Chl^{r27}, which might however be the same as Chl^{r11} and Chl^{r14}.

However since strains Chl^{r11-20} cannot be crossed together unless special markers are added by mutation, the extensive work necessary to test these conclusions on the frequency of non-allelic genes has not been carried out.

Similarly the problem of interaction between non-allelic genes was examined only to the extent that we limited our attention to the possibility of obtaining the addition, by recombination, of the resistances of independent first steps, this being a fundamental requirement of a polygenic theory.

In these experiments Chl^{r18} was crossed to Chl^{r22}, Chl^{r23}, Chl^{r25} and Chl^{r27}. These strains were selected among those which kept their resistance unimpaired for a year and were reisolated before use. Crosses were made on minimal medium and prototrophs transferred by means of the velvet technique (Lederberg, personal communication) to minimal plates containing Chl in various concentrations. Results were as given in table 7.

TABLE 7

Number of colonies grown on minimal agar with given amounts of Chl

	0γ	40γ	63γ	79γ	100γ	
$Chl^{r18} = Chl^{r22}$	132	50	22	15	8	**227**
$Chl^{r18} = Chl^{r23}$	55	37	22	2	0	**116**
$Chl^{r18} = Chl^{r25}$	443	164	13	10	8	**638**
$Chl^{r18} = Chl^{r27}$	159	73	13	12	5	**262**
	789	*324*	*70*	*39*	*21*	**1243**

The parent strains tested for resistance on complete medium showed a similar degree of resistance, both growing at 63 γ/ml. and not at 79 γ/ml. Therefore the experiments reported in the table seem to show that recombinants from independent first steps can reach a higher degree of resistance than either parent. This was checked by isolating from drug-free minimal agar the prototrophs showing apparently higher resistance, and they were found to give a resistance occasionally as high as 159 γ/ml. Since the technique of transferring whole colonies did not prove sufficiently reliable from a quantitative point of view, one of these crosses ($Chl^{r18} \times Chl^{r22}$) was repeated with the ordinary technique, *i.e.* by streaking suspensions prepared from each prototroph on plates of agar containing various concentrations of the drug (in parallel with controls, that is streaks from the sensitive parents). In two experiments, there were 3 per cent and 4 per cent of the prototrophs showing a resistance higher than that of either parent.

To control more accurately this positive interaction between independent first steps, both of them were crossed between themselves and with the (complementary) sensitive parents, *i.e.* the cross $Chl^{r18} \times Chl^{r22}$ was made in parallel with

(1) cross $Chl^{r18} \times$ the normal sensitive parent of Chl^{r22} which is #102.

(2) cross $Chl^{r22} \times$ the normal sensitive parent of Chl^{r18} which is #100.

The crosses were carried out on minimal medium in absence of Chl and the prototrophs later scored for resistance and Lac V_1 phenotype. In table 8 are shown the results obtained, *i.e.* the distribution of resistance of the recombinants from these three crosses and their distribution according to Lac V_1 phenotype.

TABLE 8

Positive interaction between independent first steps

#100 Chl^{r18} × #102 Chl^{s}

Lac	V_1	Chl resistance: <20	20	32	50	79	126	
+	s	19	25	3	7	3	0	57
−	s	75	3	3	1	0	0	82
−	r	45	1	2	1	0	0	49
+	r	3	0	0	1	0	0	4
		142	**29**	**8**	**10**	**3**	**0**	**192**

#100 Chl^{s} × #102 Chl^{r22}

Lac	V_1	<20	20	32	50	79	126	
+	s	10	4	22	10	0	0	46
−	s	5	2	50	21	0	0	78
−	r	3	3	29	15	0	0	50
+	r	1	1	3	3	0	0	8
		19	**10**	**104**	**49**	**0**	**0**	**182**

#100 Chl^{r18} × #100 Chl^{r22}

Lac	V_1	<20	20	32	50	79	126	
+	s	1	0	7	26	9	3	46
−	s	7	4	12	59	9	3	94
−	r	4	3	9	38	0	2	55
+	r	1	0	0	4	2	3	10
		13	**7**	**28**	**127**	**20**	**11**	**205**

The cross Chl^{r18} × #102 gave a small percentage of recombinants (24 out of 192, *i.e.* 11 per cent) which were of resistance as high as that of the Chl^{r18} parent (*i.e.* between 32 and 79 γ/ml.) and a majority of sensitives or slightly resistant recombinants. As it has been said before these experiments were conducted in another laboratory and with other media; therefore the absolute levels of resistance are not exactly correspondent with those seen earlier, although the relative levels are more closely correspondent.

From the distribution of resistants according to Lac V_1 markers, the gene for resistance should be located closely enough to BM and right of it.

The cross Chl^{r22} × #100 shows a majority of resistant recombinants, there being 153 out of 182, *i.e.* 84 per cent, prototrophs with a resistance level higher than 31 and lower than 79, with very little if any correlation with the Lac V_1 markers. Mapping of the gene involved is uneasy; however it is linked with BM, but not very closely, and perhaps left of it.

From the evidence of the crosses of the first step resistant with sensitive strains it would be predicted that 11 per cent × 84 per cent

$=9{\cdot}2$ per cent of the prototrophs from the cross $Chl^{r18} \times Chl^{r22}$ should have a higher resistance than either parent, and should be mostly if not all of the $Lac + V_1^s$ phenotype. The actual results gave 11 prototrophs out of 205 (5·4 per cent) which were of resistance of 126 γ/ml. and higher (that is practically double that of either resistant parent). This proportion of double-resistant recombinants (5·4 per cent) is in relatively good agreement with expectation (9·2 per cent) especially if one considers that the limits used for distinguishing between sensitives, resistants and double-resistants (less than 31, between 31 and 79, more than 79 γ/ml.) are arbitrary and at least some resistants to 79 γ/ml. of the $Chl^{r18} \times Chl^{r12}$ cross might have been double-resistants, thus increasing the proportion of these by some units per cent. The expectation according to phenotype of double-resistants is at least partly confirmed, if one considers that of the 11 double-resistant prototrophs 6 show recombination, as expected, in the 1st region and 5 in the other two regions on a total of 55 recombinations in the 1st region (M – Lac) to 168 recombinations in the 2nd and 3rd ($Lac - V_1$ and $V_1 - TL$).

6. DISCUSSION

It is possible to obtain higher and higher resistance to Chloromycetin in *E. coli* K-12 by repeated transfers on growing concentrations of the drug, while a single exposure will result in the isolation of partially resistant individuals. The resistance acquired is relatively stable on subsequent cultivation in drug-free media but on prolonged culture it will often be lost unless special precautions are taken. However, loss of resistance requires months or years and the resistance levels are stable enough for short-term experiments.

This phenomenon of gradual adaptation, which is well known for many other drugs and micro-organisms, can be analysed genetically in the present case.

In principle the adaptation to Chl could be the result of one (or both) of the following mechanisms:

(i) physiological adaptation, by which resistance is developed gradually, the levels being comparable with the concentrations of drug employed during training. Hinshelwood (see for instance Dean and Hinshelwood) has proposed a model of gradual adaptation which could *a priori* explain these results, provided one assumes that the changes in the enzyme-systems determined by contact with the drug, and which make the cells capable of withstanding the higher drug concentrations, are heritable in the absence of the drug for hundreds or thousands of generations;

(ii) a mutation and selection mechanism, in which case one can explain the existence of various steps of resistance by assuming

(*a*) a system of multiple alleles entailing various degrees of resistance;

(*b*) or preferably a polygenic system, which would more reason-

ably account for the other fundamental aspect of this phenomenon, that in fact high resistance is never reached in a single step.
Mechanisms (*a*) and (*b*) can, however, co-exist.

Mechanism (*b*) was suggested by Demerec when he described the stepwise adaptation of *Staphylococcus aureus* to Penicillin.

Present experiments do not offer a direct answer to the question whether adaptation is of the physiological type (as assumed by Hinshelwood for other cases of drug resistance) or genetical, *i.e.* by mutation and selection. This question has been answered with more direct experiments (Cavalli, 1952) which were in favour of the second theory, thus confirming previous evidence for other drugs and organisms (Demerec, 1945; Oakberg and Luria, 1947; Newcombe and Hawirko, 1949; and others).

The present experiments however give a strong indirect support to the genetical theory, in that they show that wherever resistance is acquired, genetical analysis—by crossing to sensitives—shows segregations of gene-differences due to loci linked with the ordinary markers. Moreover, highly resistant strains show, on crossing to sensitives, all possible intermediate degrees of resistance, as expected if many genes are involved. When strains with intermediate resistance, obtained with one short exposure to the drug, are crossed to sensitives, the segregations obtained denounce only one or at most two gene-differences.

However, the point which is most strongly in favour of the polygenic theory is that such genes are usually linked with the ordinary chromosome markers.

A finer analysis of segregation from first steps shows that even when a small degree of resistance is involved, there is no inheritance of the blending type.

On crossing independent first steps together as well as crossing high resistants to sensitives, it is found that some 5 and probably more loci are involved.

As expected under the polygenic theory, resistance-genes which are not allelic (as shown by (i) the fact that when crossed together they give rise to a certain proportion of sensitives and also (ii) if crossed to marked sensitives they show different linkages with the markers) can on recombination add their effects, giving recombinants with a resistance clearly higher than that of either parent.

Therefore while these results are in good agreement with a polygenic theory they would not be easily explained on a physiological theory.

Some facts which may at first sight cause doubt on this interpretation will be discussed in detail:

(i) resistant strains are not very stable in the long run. This may seem like " deadaptation " in a system of physiological adaptation, but it must be observed that the loss of resistance occurs only after repeated passages and is relatively abrupt, which is rather in agreement with the

hypothesis of loss of resistance by selective advantage of back mutants.

It must be stressed that a selective advantage of back mutants is to be expected, because resistant organisms grow poorly both on ordinary media and on media containing the drug: highly resistant organisms reach only 1/4 to 1/2 the saturation density which the normal parents will reach in the same conditions.

In order to keep resistant strains at the original level of resistance it is advisable to isolate at frequent intervals on drug-free media and test individual colonies for resistance, subculturing only from those which show the original level of resistance. If this is not done at frequent intervals it is likely that all the colonies tested will be of a lower level; on the other hand a selection on media containing the drug might introduce new unwanted genes for resistance.

(ii) In some of the crossing experiments no clear cut or no easily explainable linkage with the ordinary markers was found. This may mean that the loci involved are outside the marked region. A discussion of such results will however be premature, until the genetical system of *E. coli* K-12 is more fully known.

(iii) There are several facts showing that selection favours positive interaction between genes, and recombination reveals negative interaction between them. Especially the first consideration is just to be expected. In fact selection builds a resistant polygenic system in this sequence:

$$r^{\circ}\ (\text{sensitive}) < r^1 < r^1r^2 < r^1r^2r^3 \ldots$$

where $<$ and $>$ stand for less or more resistant, and the following considerations are pertinent:

(*a*) this system is compatible with

$r^2r^3 = r^{\circ}$ (negative interaction between r^2, r^3 if r^2, $r^3 > r^{\circ}$)

or even $r^2 = r^{\circ}$, $r^3 = r^{\circ}$ (r^2, r^3 "modifiers" of r^1).

(*b*) of several second step mutants r^2, r^3, r^4 which may appear in a partially resistant clone r^1, the double mutant favoured and therefore selected will be the one showing the *highest* positive interaction.

Therefore, when a polygenic system for resistance is broken down by recombination with a sensitive strain, resistance is expected to be low on average in the recombinants, and if two independently built polygenic systems are allowed to recombine together, positive interactions will disappear and negative interactions may be revealed with the result that resistance of recombinants will diminish on average.

If the results obtained by crosses of Chl resistant organisms could be generalised, one should think that the description of a polygenic system might require a number of parameters equal to the number of degrees of freedom involved; *i.e.* the number of possible genotypes minus one. This seems unfortunate but may be unavoidable. On the other hand, in the case of Chl^{r18} one locus seems to interact favourably

with all other genes, with which it was brought together by recombination. If the interactional behaviours were at least for some genes a property of genes rather than of gene combinations, the number of parameters needed to describe a polygenic system might be reduced. As far as we know we can only say that such a number of parameters will be between a minimum, which is the number of loci n involved in the polygenic system under consideration (and would be enough for a system where all interactions were additive on some suitable scale) and a maximum, which is $2^n - 1$ for a haploid organism.

(iv) Selection for one character is known to be often accompanied by changes in other characters (Mather's "correlated response"). Although here no clear cut correlation between resistance and other morphological and biochemical characters has been found, it is worth mentioning some exceptions: in one case a first step resistant was found, giving highly disturbed segregations of the ordinary markers (an excess of $Lac + V_1^r$). This may mean a chromosome mutation, or a mutation for auxotrophism accompanying Chl-resistance (not further tested). In other cases less striking disturbances of the segregations have been observed (see for instance a deficiency of $Lac - V_1^s$ recombinants in table 2, cross I) and may have similar origins.

One typical morphological change observed in most if not all first or higher step resistants, is a mucoid appearance of the resistant colonies especially when grown on media rich in carbohydrates. There are various degrees of mucosity up to giant mucoid. Recombination between a mucoid resistant and a normal sensitive gives rise to segregation into four classes, of which mucoid sensitives and normal resistants are rarest. This is a genetical puzzle which has not been solved: no serious attempts were made, however, because of the highly variable expression of the mucoid character.

These results suggest that drug resistance in micro-organisms can offer an excellent field of study for quantitative inheritance. In fact, in bacteria, unlike higher organisms, single mutational steps can be isolated and analysed genetically. The possibility of analysis in biochemical terms of the effect of single genes is readier to hand, thus making an interpretation of interactions between different genes more easily attainable. However for such an analysis three conditions at least must be met: a fuller knowledge of the genetical system of bacteria, a drug with mechanism of action better known than that of Chl, and an accurate and simple method for measuring resistance.

7. CONCLUSIONS AND SUMMARY

1. Resistance to Chl (Chloromycetin) in *E. coli* K-12 is acquired only gradually. Short exposures secure only a moderate degree of resistance, repeated selection being necessary to reach high levels.

2. The genetical analysis has shown that a polygenic system is involved. In fact (i) on crossing high resistants to sensitives one finds recombinants showing all degrees of resistance between those of the

parents; (ii) polygenes determining resistance are linked with the ordinary chromosome markers.

3. Recombinations between non-allelic independent first step resistants can result in addition of their resistances, so that prototrophs arise which are more resistant than either parent.

4. Repeated selection tends to build a polygenic system rich in positive interactions. Recombination is likely to break down such positively interacting systems and, by bringing together resistance genes which need not interact positively or even simply additively, may reveal important negative interactions.

5. These results are not easily compatible with a theory of physiological adaptation.

Acknowledgment.—We wish to take this opportunity to thank Professor R. A. Fisher for his interest and encouragement during this investigation.

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Note added in proof.—While this paper was in the press, a report of work by R. D. Hotchkiss was published (*Cold Spring Harbor Symposia*, vol. XVI) showing that the transfer of heritable characters like various drug resistances can take place in *Pneumococci* by exposing in suitable conditions sensitive cells to cell-free, DNA-containing preparations obtained from resistant cells. While this has greatly amplified the meaning of the early transformation experiments in *Pneumococci*, it has made genetic analysis possible in essentially asexual organisms. In particular, the results given by Hotchkiss on the inheritance of penicillin resistance fit satisfactorily the scheme which we have also used to explain chloromycetin resistance.

LE RÔLE DES FLUCTUATIONS FORTUITES DANS LA DIVERSITÉ DES POPULATIONS NATURELLES DE *CEPAEA NEMORALIS* (L.)

M. LAMOTTE
Laboratoire de Génétique de la Faculté des Sciences, Paris

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1. INTRODUCTION

L'ATTENTION a été attiré, depuis quelques années, sur un facteur longtemps négligé dans l'étude des phénomènes évolutifs: la variation fortuite des fréquences géniques, liée à l'effectif forcément limité des populations naturelles. S. Wright (1931, 1935) a montré, par des développements mathématiques, le rôle de cette " dérive génétique " (" genetic drift "), notamment dans l'évolution de populations de faible effectif. D'autres auteurs, au contraire, paraissent lui attribuer une bien moindre importance, sinon même lui dénier tout rôle, en regard des forces sélectives (Fisher et Ford, 1947; Cain et Sheppard, 1950). Mais bien peu d'exemples, au total, ont été donnés pour appuyer ces vues surtout théoriques, et leur interprétation prête encore souvent à discussion (Fisher et Ford, 1947; Wright, 1948). Ce sont en effet des centaines de générations qu'exigerait une expérimentation valable dans ce domaine, c'est-à-dire des dizaines d'anées au moins pour des animaux comme la Drosophile, dont le cycle vital est pourtant rapide.

Devant l'impossibilité de réaliser de telles expériences, nous avons cherché à transposer l'étude de la variation du plan temporel dans le plan spatial, en interprêtant les résultats fournis par l'examen simultané d'un ensemble de populations actuelles. Plus précisément, nous avons analysé les rapports existant entre le facteur qui est à la base même des fluctuations au hasard, la grandeur de l'effectif, et la diversité de composition génique des populations. Si cette diversité a pour origine des *différences dans le niveau d'équilibre des gènes*, différences qui peuvent d'ailleurs être liées à des facteurs internes—variété des compositions géniques globales des colonies—aussi bien qu'à des facteurs externes —actions divergentes du milieu environnant—, elle ne doit pas être influencée par la grandeur de l'effectif. Dans la mesure, au contraire, où interviennent des *fluctuations fortuites*, celles-ci auront un rôle d'autant plus marqué que l'effectif est plus faible: la diversité des fréquences géniques sera plus accentuée parmi les petites populations que parmi les grandes.

Une espèce nous a paru se prêter tout spécialement à une telle étude: l'Escargot des bois, *Cepaea nemoralis* (L.), qui présente le double

avantage d'être polymorphe et de vivre en populations bien limitées (Lamotte, 1951). Nous envisagerons plus particulièrement ici deux traits de ce polymorphisme dont le déterminisme génétique est connu (Lang, 1904, 1909, 1912; Lamotte, 1951): l'absence ou la présence de bandes pigmentées sur la coquille (gènes b_+ et b) et la coloration rose ou jaune de cette coquille (gènes j + et j).

2. ETUDE EXPÉRIMENTALE DE L'INFLUENCE DE L'EFFECTIF SUR LA DISPERSION DES FRÉQUENCES GÉNIQUES

Malgré les nombreuses recherches consacrées à l'interprétation des modalités du polymorphisme chez *Cepaea nemoralis* (Schilder, 1925; Boettger, 1931; Schnetter, 1950; Cain et Sheppard, 1950), les données relatives à la composition génique des populations naturelles de l'espèce et, plus encore, à la grandeur de leur effectif, restaient encore très insuffisantes. De nombreuses colonies ont donc été étudiées dans toutes les régions de France, et leur composition déterminée à partir d'un échantillon aussi important que possible (Lamotte, 1951). Pour plusieurs centaines d'entre elles, on a pu également estimer l'effectif des escargots adultes, soit par la méthode des marquages et recaptures, soit par dénombrement direct des coquilles gisant sur le sol après brûlis des buissons où vivait la colonie, soit encore par comparaison avec des populations étudiées par l'une des deux méthodes précédentes. Nous considérerons ici plus particulièrement les deux catégories extrêmes de colonies: celles à effectif restreint—compris entre 500 et 1000 individus—, et celles à effectif élevé—entre 3000 et 10.000 individus.

En ce qui concerne le gène inhibiteur de bandes (b +), par exemple, les résultats suivants ont été observés (voir fig. 1):

Fréquence du gène b +	0, -0, 10	0, 10 -0, 20	0, 20 -0, 30	0, 30 -0, 40	0, 40 -0, 50	0, 50 -0, 60	0, 60 -0, 70	0, 70 -0, 80	0, 80 -0, 90	0, 90 -1
colonies de 500 à 1000 ind.	53 (5)	23	10	9	5	4	2	—	—	3 (3)
colonies de 3000 à 10.000 ind.	46 (5)	13	11	4	1	1	1	—	—	—

Les dispersions de ces deux distributions sont mesurées respectivement par les variances*:

$$V_p = 35 \times 10^{-3}$$
$$V_g = 16 \times 10^{-3}$$

c'est-à-dire que la dispersion des fréquences du gène " absence de bandes " est plus de deux fois supérieure dans les colonies de faible effectif.

* Les variances ont été calculées directement à partir des fréquences observées dans les populations, pour éviter l'inexactitude qu'introduirait le groupement.

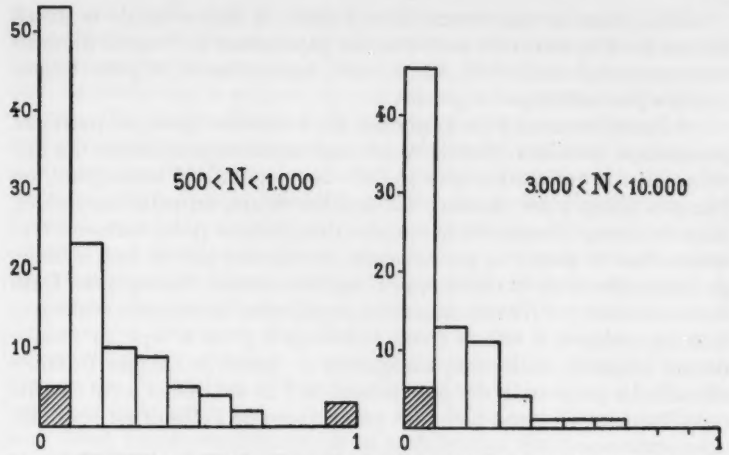

FIG. 1.—Distribution des fréquences du gène inhibiteur de bandes (b_+) dans les populations de faible et de fort effectif de *Cepaea nemoralis*.

Des résultats semblables se retrouvent dans le cas du gène j, déterminant la coloration jaune de la coquille (voir fig. 2):

Fréquence du gène j	0, -0, 10	0, 10 -0, 20	0, 20 -0, 30	0, 30 -0, 40	0, 40 -0, 50	0, 50 -0, 60	0, 60 -0, 70	0, 70 -0, 80	0, 80 -0, 90	0, 90 -1
colonies de 500 à 1000 ind.	6 (2)	2	3	5	7	8	7	8	9	7 (5)
colonies de 3000 à 10.000 ind.	0	3	4	6	5	7	6	5	5	8 (2)

Les dispersions correspondantes sont:

$$V_p = 53 \times 10^{-3}$$
$$V_g = 29 \times 10^{-3}$$

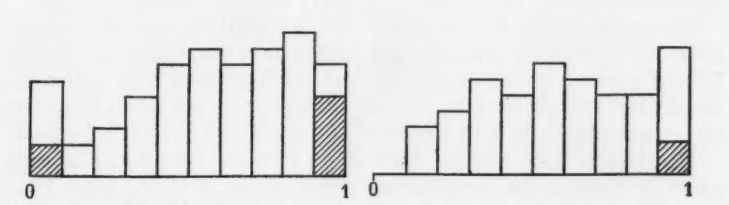

FIG. 2.—Distribution des fréquences du gène jaune (j) dans les populations de faible et de fort effectif de *Cepaea nemoralis*.

Ainsi, dans un cas comme dans l'autre, la dispersion de la distribution des fréquences du gène dans les populations de l'espèce diminue très nettement lorsque l'effectif s'accroît. Autrement dit, *les petites colonies sont bien plus variées que les grandes.*

A l'accroissement de la dispersion des fréquences géniques parmi les populations de faible effectif s'ajoute une autre caractéristique des distributions: la proportion plus grande des populations homogènes, où l'un des allèles a été éliminé. Ce nombre figure, en petits caractères, dans les classes marginales des quatre distributions précédemment étudiées. Pour le gène b+, par exemple, on constate que les trois colonies de faible effectif de la classe 0,90-1 sont des colonies homogènes. Dans le cas du gène j, il n'existe que deux populations homogènes seulement dans la catégorie à effectif élevé, tandis qu'il y en a sept au total—deux à fréquence nulle, cinq à fréquence 1—parmi les colonies de faible effectif. La proportion des populations où l'un des allèles a été éliminé complètement est donc bien plus grande lorsque l'effectif est restreint.

3. ETUDE THÉORIQUE DES RAPPORTS ENTRE L'EFFECTIF ET LES FLUCTUATIONS FORTUITES

Ainsi, l'observation d'un grand nombre de populations permet de constater que la diminution de l'effectif va de pair avec un net accroissement de la dispersion des fréquences géniques, se manifestant par un nombre plus élevé de populations homogènes aussi bien que par l'augmentation de la variance de l'ensemble de la distribution. Ces faits ne peuvent guère s'expliquer que par l'intervention de fluctuations purement fortuites, dont ils sont la conséquence directe. En effet, si la fréquence d'un gène était, dans chaque colonie, déterminée strictement par l'ensemble des facteurs externes—les conditions du milieu—et des facteurs internes—le patrimoine génétique global de la colonie—, l'effectif n'interviendrait en rien dans le niveau d'équilibre du gène, dont la distribution parmi les petites colonies serait, par conséquent, identique à sa distribution parmi les grandes colonies.

Il est intéressant de considérer la question sous son aspect quantitatif et de rechercher l'importance relative des fluctuations fortuites ainsi mises en évidence et des autres causes possibles de diversité des fréquences géniques, en particulier le rôle des facteurs externes, généralement mis en avant par les auteurs qui ont abordé déjà l'étude de *Cepaea nemoralis* (Schilder, 1925; Boettger, 1931; Cain et Sheppard, 1950).

La comparaison des variances des distributions apporte évidemment à la solution du problème une information quantitative précieuse. Mais il importe de remarquer que son interprétation n'est nullement immédiate: elle nécessite en effet de se reporter à un schéma théorique du comportement du gène considéré et d'y analyser le rôle de l'effectif.

Imaginons donc que les fluctuations fortuites constituent la seule cause de diversité des compositions géniques: on se trouve alors placé dans les conditions admises dans le schéma de S. Wright (1931), c'est-à-dire qu'il est possible, connaissant les taux de mutation de deux allèles

et les taux de sélection des trois génotypes correspondants, de déduire la distribution des fréquences géniques dans les diverses populations de l'espèce, supposées toutes de même effectif. Il suffirait donc, en principe, de considérer le rapport des variances de " courbes de Wright " correspondant à des effectifs différents des colonies, toutes choses étant égales d'ailleurs: si ce rapport est égal à celui que fournissent les résultats expérimentaux, les fluctuations fortuites suffisent à expliquer la diversité observée; sinon, il faut admettre que des causes supplémentaires de diversité, que ne prévoient pas les hypothèses de Wright, viennent s'ajouter aux fluctuations fortuites, et leur importance sera d'autant plus grande que les rapports des variances différeront davantage.

Examinons d'abord un cas simplifié, où l'équilibre est déterminé par les seules mutations, directe et réverse, et par la migration, dont l'action peut être assimilée à celle de mutations (S. Wright, 1931). Dans ce cas, la relation de passage d'une génération à la suivante est linéaire et de la forme $\delta q = v - (u+v)q$, et la théorie de Wright conduit, pour l'état limite stationnaire de la distribution des fréquences, aux relations:

$$\text{moyenne} = M = \frac{v}{u+v}, \text{ indépendante de l'effectif } N \text{ des colonies;}$$

$$\text{variance} = V = \frac{uv}{(u+v)^2} \quad \frac{1}{4N(u+v)+1} = M(1-M)\frac{1}{4N(u+v)+1}$$

Pour deux distributions correspondant à des colonies ne différant que par leurs effectifs N_1 et N_2, on a donc:

$$\frac{V_1}{V_2} = \frac{4N_2(u+v)+1}{4N_1(u+v)+1}$$

Lorsque les termes $4N(u+v)$ sont sensiblement supérieurs à 1—ce qui est le cas pour des distributions " en cloche "—, on voit que la variance est à peu près inversement proportionnelle à l'effectif N des colonies, dans l'hypothèse faite où les fluctuations fortuites déterminent seules la diversité des fréquences géniques dans les populations de l'espèce.

Une telle comparaison analytique cesse malheureusement d'être possible dans le cas général où l'équilibre génique fait intervenir, outre les taux de mutation, des taux de sélection quelconques des trois génotypes en présence: ce qui est le cas, sans doute le plus fréquent, d'un polymorphisme " balancé ", où l'hétérozygote possède une vitalité supérieure aux deux homozygotes. En effet, l'effectif intervient alors dans des relations transcendantes complexes dont on ne peut trouver de solution simple. On peut seulement affirmer que la variance se rapproche d'autant plus de zéro que l'effectif est plus grand.

Il est possible, toutefois, d'étudier numériquement le problème dans tous les cas particuliers que l'on désire, en construisant point par point chaque distribution théorique:

$$f(q) = Kq^{4Nv-1}(1-q)^{4Nu-1}\exp.\{2Nwq^2 + 4Ntq\}$$

et en déterminant graphiquement sa variance. Sans détailler les calculs, d'ailleurs fort laborieux, qui sont nécessaires, nous nous bornerons à exposer ici les résultats obtenus pour un certain nombre de valeurs des paramètres relatives à des courbes de Wright qui se rapprochent sensiblement de celles observées expérimentalement.

Les paramètres adoptés en premier lieu sont les meilleures estimations correspondant à la distribution du gène b+ réellement observée dans 280 populations naturelles d'Aquitaine (Lamotte, 1951): les taux de mutation et de sélection u, v, w et t* sont égaux respectivement à $u=24\times10^{-3}$, $v=0$, 6×10^{-3}, $w=44\times10^{-3}$ et $t=18\times10^{-3}$. On a pris successivement pour l'effectif des colonies les valeurs 500, 1000, 2000, 4000 et 8000; ce sont les courbes théoriques ainsi définies que reproduit la figure 3*a*.

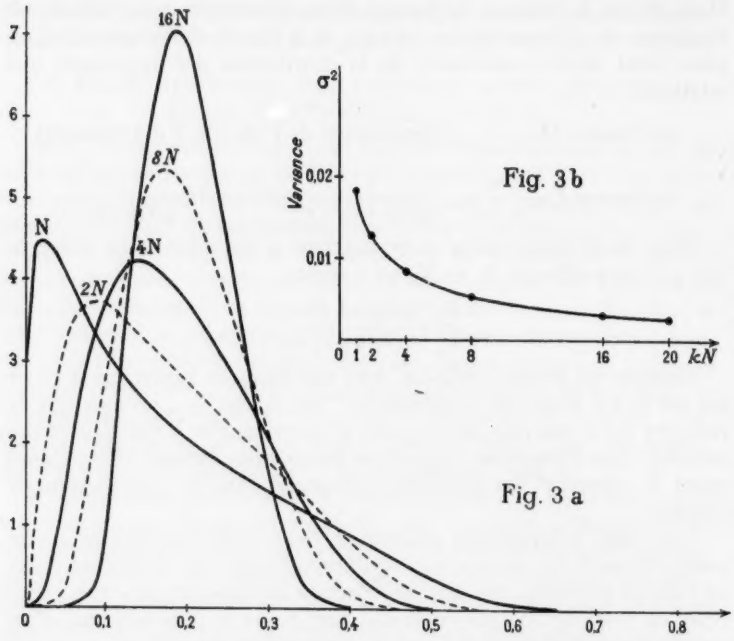

FIG. 3A.—Courbes de Wright correspondant aux paramètres de mutation et de sélection $u=24\times10^{-3}$, $v=0$, 6×10^{-3}, $w=44\times10^{-3}$, $t=18\times10^{-3}$, et aux différents effectifs $N=500$, $2N=1000$, $4N=2000$, $8N=4000$ et $16N=8000$.

FIG. 3B.—Loi de variation, en fonction de l'effectif, des variances des courbes de Wright correspondant aux paramètres de 3a.

* Voir Malécot (1948) et Lamotte (1951). Les paramètres u et v traduisent les effets conjoints de la mutation et de la migration.

Quant aux paramètres de sélection w et t, introduits par Malécot, ils sont définis par les expressions $w=\frac{\sigma_1-2\sigma_2+\sigma_3}{\sigma_1}$ et $t=\frac{\sigma_3-\sigma_1}{\sigma_1}$, où σ_1, σ_2 et σ_3 sont les valeurs sélectives respectives de l'homozygote récessif, de l'hétérozygote et de l'homozygote dominant.

Le tableau qui suit indique les valeurs des variances de ces distributions, dont la figure 3*b* représente graphiquement la loi de variation en fonction de l'effectif des colonies.

Effectif	500	1000	2000	4000	8000
Variance	18, 5 $\times 10^{-3}$	12, 9 $\times 10^{-3}$	8, 4 $\times 10^{-3}$	5, 2 $\times 10^{-3}$	3, 1 $\times 10^{-3}$

Deux autres familles de distributions théoriques ont été étudiées, dont chacune correspond à des valeurs déterminées des paramètres de mutation et de sélection et, comme précédemment, aux divers effectifs 500, 1000, . . . 8000.

La première de ces deux séries a pour paramètres: $u = 5 \times 10^{-3}$, $v = 0$, 5×10^{-3}, $w = 0$ et $t = 5 \times 10^{-3}$. On trouve, pour les variances des diverses distributions:

Effectif	500	1000	2000	4000	8000
Variance	23, 5 $\times 10^{-3}$	13, 5 $\times 10^{-3}$	7, 3 $\times 10^{-3}$	3, 8 $\times 10^{-3}$	2, 0 $\times 10^{-3}$

Pour la seconde série, les paramètres sont: $u = 5 \times 10^{-3}$, $v = 0$, 5×10^{-3} $w = 0$ et $t = 2$, 5×10^{-3}. Les variances trouvées, correspondant aux divers effectifs sont alors:

Effectif	500	1000	2000	4000	8000
Variance	13, 1 $\times 10^{-3}$	7, 6 $\times 10^{-3}$	4, 2 $\times 10^{-3}$	2, 3 $\times 10^{-3}$	1, 4 $\times 10^{-3}$

Ces quelques applications numériques à des distributions théoriques de S. Wright donnent une idée de la décroissance de la variance de ces distributions lorsqu'augmente l'effectif des populations. Elles ne permettent pas de déterminer l'expression d'une loi analytique commune, qui dépend d'ailleurs des divers paramètres, mais il apparait que, dans les cas étudiés, la variance décroît plus lentement que l'inverse de l'effectif. Plus précisément, ces résultats montrent que le rapport des variances correspondant aux effectifs N_g (4000 individus en moyenne) et N_p (entre 500 et 1000 individus) des deux groupes de colonies de *C.n.* étudiés plus haut, est compris entre 1/3 et 1/5; quant à la variance de la distribution des colonies de fort effectif (N_g), on voit qu'elle est comprise entre le quart et la moitié de la différence des variances des deux groupes.

4. INTERPRÉTATION DES RÉSULTATS OBSERVÉS

Revenons maintenant aux résultats observés pour les gènes " jaune " et " absence de bandes " dans les populations de *Cepaea nemoralis*.

Pour le gène " jaune ", tout d'abord, nous avons vu que la variance passait de 53×10^{-3} dans la distribution des petites colonies à 29×10^{-3} dans celle des grandes, dont l'effectif est, en moyenne, quatre à six fois plus élevé. Or, d'après les lois théoriques qui viennent d'être exposées, le rapport de ces variances devrait être notablement plus fort et dépasser 3 ou 4: il faut en conclure que les fluctuations fortuites ne sont pas seules à l'origine de la diversité des fréquences géniques et que d'autres causes, indépendantes de l'effectif des populations, interviennent également. On peut remarquer, d'ailleurs, que les variances sont anormale-

ment élevées pour être simplement dues à des fluctuations fortuites. Mais il n'en reste pas moins que, dans le cas des petites colonies, d'effectif inférieur à un millier d'individus, la diversité due aux fluctuations fortuites doit représenter, mesurée par la variance des distributions, la moitié au moins de la diversité globale, puisqu'elle diminue de moitié lorsque l'effectif s'accroît.

Chez les populations d'effectif élevé, la part de la dérive génétique dans la diversité observée ne peut plus être évaluée directement comme ci-dessus, puisqu'il serait nécessaire de considérer la dispersion génique parmi des populations d'effectif infini, qui n'existent évidemment pas. L'examen des lois théoriques de variation de la variance en fonction de l'effectif permet toutefois d'en rechercher une approximation.

Nous avons remarqué, en effet, que la variance fortuite donnée par les formules de Wright était comprise, pour les colonies d'effectif voisin de 4000, entre le quart et la moitié de la différence des variances relatives aux distributions des grandes et des petites colonies. On peut donc estimer que la part de la dispersion génique qui revient aux fluctuations fortuites, dans le cas des grandes colonies, correspond à une variance supérieure à $\frac{53 \times 10^{-3} - 29 \times 10^{-3}}{4} = 6 \times 10^{-3}$. Comme la variance totale est 29×10^{-3}, la part des fluctuations fortuites en représente au moins le cinquième.

Dans le cas des petites colonies, la part des fluctuations fortuites dépasse donc $(53 \times 10^{-3} - 29 \times 10^{-3}) + 6 \times 10^{-3}$ soit 30×10^{-3}, c'est-à-dire plus de la moitié de la variance globale.

Le reste de la diversité semble devoir être attribué à l'action du milieu, différent selon les biotopes, comme nous aurons l'occasion de la montrer ultérieurement.

Dans le cas du gène " absence de bandes ", la variance de la distribution des petites colonies est sensiblement plus de deux fois supérieure à celle de la distribution des grandes: 35×10^{-3} au lieu de 16×10^{-3}. Le rôle des fluctuations fortuites est donc plus important encore que précédemment.

Pour les populations d'effectif élevé, la part de la dispersion imputable à ces fluctuations peut être estimée, de la même façon que pour le gène " jaune ", au quart au moins de la différence entre les variances des distributions des petites et des grandes colonies, soit ici $\frac{35 \times 10^{-3} - 16 \times 10^{-3}}{4} = 5 \times 10^{-3}$. Pour les petites colonies, la dispersion due aux fluctuations fortuites dépassera donc $(35 \times 10^{-3} - 16 \times 10^{-3}) + 5 \times 10^{-3} = 24 \times 10^{-3}$, c'est-à-dire les deux tiers de la variance totale qui est 35×10^{-3}.

Il convient même de remarquer que la part de la dérive génétique apparaîtrait relativement bien plus forte encore dans le cadre d'une région limitée et non plus dans l'ensemble de toutes les régions de France. Il existe en effet des divergences régionales importantes dans

la fréquence moyenne d'équilibre du gène " absence de bandes " (Lamotte, 1951). Ces divergences sont sans rapport aucun avec les facteurs externes, climatiques ou autres, et dues à l'isolement relatif des populations: c'est le faible pouvoir de déplacement des individus qui a amené la ségrégation de races locales élémentaires, différant par le niveau moyen d'équilibre des principaux gènes du polymorphisme. Une telle variabilité locale doit naturellement accroître de façon identique la dispersion des fréquences géniques parmi les grandes et parmi les petites colonies, qui proviennent les unes et les autres de l'ensemble des provinces.

Une étude faite dans une sule région aurait permis, en éliminant ce facteur régional de diversité, de confronter seulement les rôles respectifs des fluctuations fortuites et des différences d'équilibre sélectif liées à la diversité des biotopes; la nécessité de considérer un nombre de populations suffisamment élevé n'a pas permis de la réaliser. Il est toutefois possible d'estimer la part qui, dans la diversité génique de l'ensemble des colonies, revient à l'hétérogénéité des stocks régionaux: il suffit de déterminer la variance de la distribution des fréquences géniques régionales. Ces fréquences moyennes* du gène " absence de bandes " sont, dans les principales régions d'où proviennent les populations étudiées:

Aquitaine	0, 19	Somme	0, 16
Pyrénées	0, 15	Alpes	0, 17
Bretagne	0, 16	Centre	0, 11
Région Paris	0, 12	Rég. Rhod.	0, 32
Nord	0, 07	Mass. Centr.	0, 09
Est	0, 29	Ouest	0, 11

d'où la variance d'hétérogénéité régionale:

$$V_{hét} = 6 \times 10^{-3}$$

On peut donc estimer que, *dans le cadre d'une région limitée*, les dispersions du gène " absence de bandes " seraient respectivement, dans les petites et dans les grandes colonies†:

$$V_p = 35 \times 10^{-3} - 6 \times 10^{-3} = 29 \times 10^{-3}$$
$$V_g = 16 \times 10^{-3} - 6 \times 10^{-3} = 10 \times 10^{-3}$$

Dans ces conditions, la part de variance revenant aux fluctuations fortuites, que nous avons trouvée égale à 24×10^{-3} pour les colonies de faible effectif et à 5×10^{-3} pour les colonies d'effectif élevé, représente, pour les premières, les 5/6 de la variance totale et, pour les autres, elle en est encore la moitié.

5. CONCLUSIONS

Les fluctuations fortuites—ou dérive génétique—sont donc à l'origine d'une part importante de la diversité que l'on observe parmi les

* Dont chacune a été déterminée à partir de plusieurs dizaines de colonies.

† Une bonne confirmation en est apportée par les valeurs des variances réellement observées pour l'ensemble des colonies, de tous effectifs, dans chacune des régions : ces variances sont en effet comprises entre les deux valeurs précitées. En Aquitaine, par exemple, $V = 13 \times 10^{-3}$; dans la région parisienne, $V = 15 \times 10^{-3}$; dans les Pyrénées, $V = 14 \times 10^{-3}$; en Bretagne, $V = 20 \times 10^{-3}$.

fréquences géniques dans les populations naturelles de *C.n.* Il faut voir là, sans doute, une conséquence directe de l'effectif en général très limité des colonies de cette espèce, et cela d'autant plus que l' " effectif efficace " (" effective size ") est certainement bien plus réduit encore: l'instabilité des biotopes habituels de l'espèce—biotopes " secondaires ", liés à l'action de l'homme—limite en effet la durée d'existence de la plupart des colonies et accroît par là l'importance relative des premières années de la colonie, où l'effectif est naturellement très restreint et où, par conséquent, les fluctuations au hasard peuvent jouer un rôle primordial.

Mais il faut considérer aussi ce rôle important de la dérive génétique dans la distribution des fréquences de certains gènes—et c'est plus particulièrement le cas, ici, du gène " absence de bandes "—comme la marque d'un équilibre génique déterminé sans grande rigueur, c'est-à-dire lié à des valeurs sélectives de très faible intensité.

Il serait évidemment hasardeux de vouloir généraliser de telles conclusions à n'importe quel autre gène et, plus encore, à n'importe quelle autre espèce, de même qu'il n'est guère admissible de dénier systématiquement tout rôle aux fluctuations fortuites, dont l'existence est une conséquence inéluctable de l'effectif limité des populations naturelles. Il est évident que chaque espèce et, dans chacune d'elle, chaque gène, constitue un cas particulier qu'il convient d'étudier en détail si l'on veut pouvoir conclure à son endroit: la comparaison des cas respectifs du gène " jaune " et du gène " absence de bandes " montre bien cette diversité de comportement à l'intérieur d'une même espèce et l'examen d'autres gènes ne ferait que confirmer ce point de vue.

D'une façon très générale, la part de la dérive génétique sera d'autant plus importante dans la dispersion des fréquences d'un gène que les niveaux d'équilibre géniques seront moins strictement déterminés. Dans le cas, assez rare sans doute dans la nature, où n'existe, dans les diverses populations de l'espèce, qu'un seul niveau d'équilibre pour un gène déterminé—c'est-à-dire, pratiquement, lorsque cet équilibre est indépendant des conditions de milieu—, les distributions théoriques de S. Wright donnent directement une image quantitative du phénomène. Dans les autres cas, elles permettent de rechercher une estimation des rôles des différents facteurs de diversité, ainsi qu'il a été fait pour *C.n.*

6. ENGLISH SUMMARY

The polymorphism of *Cepaea nemoralis* (L.) is characterised by an extreme variation in the frequencies of varieties in different natural populations. The author has set out to determine the role of fortuitous fluctuations (genetic drift) in this diversity.

Toward this end, a comparative study was made of the scatter of the frequencies of genes b+ and j in two categories of colonies: those composed of many individuals (over 3000) and those composed of few (less than 1000). If the gene frequencies were in all instances rigorously determined by the interplay of selective forces, their distribution would

be independent of the number of individuals in the colonies. In fact, this proves not to be the case, for variation is considerably greater among the small populations than it is among the big. Thus, genetic drift must play an important role in the diversity of the gene frequencies of these populations, for it is the only factor which depends upon population size.

The author has tried to determine quantitatively the portion of variation which is due to genetic drift, in view of the difference in the values of genic equilibria in the various environments. In order to do this, a study was made of the expected variation of the scatter as a function of population size, assuming that genetic drift alone operated. This was done by determining the variance of Wright's curves corresponding to different colony sizes. The relationship of the variances actually observed in the two categories of colonies was compared with the theoretical law of variation thus estimated.

It appears that genetic drift has a less appreciable role in determining variation of yellow and pink forms, whereas its role is preponderant in determining variation of banded and unbanded forms. In the case of the latter, even in large colonies genetic drift is responsible for half of the scatter observed within a given limited region. In small colonies, which are the most frequent, fluctuations resulting from genetic drift represent 5/6 of the total scatter.

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MULTIPLE SEX-CHROMOSOMES IN THE MARSUPIAL, *POTOROUS*

G. B. SHARMAN AND H. N. BARBER
Department of Botany, University of Tasmania

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1. INTRODUCTION

SINCE 1922 the cytology of twenty-seven species of marsupials has been investigated (table 1). Chromosome numbers in this group are, in general, much lower than those of eutherian mammals and the large size of the chromosomes makes the group favourable for cytological studies. In most of the species studied a typical XY sex-determination mechanism has been reported. Koller (1936) showed that the pairing segments of the X and Y chromosomes are terminal or nearly so, and that the centromeres are included in the differential segments. The first meiotic division is therefore reductional. In a preliminary account (Sharman, McIntosh and Barber, 1950) we have shown that in the macropod marsupial *Potorous tridactylus* three chromosomes in the male are concerned with sex determination and form a trivalent at meiosis. The female has one chromosome less than the male. There appears to be a comparable case in another macropod marsupial *Wallabia bicolor* (*Macropus ualabatus*) studied by Agar (1923). Agar considered that the XY bivalent in this species was " possibly sometimes independent but more often attached to one of the autosomes ". Agar's work has been reinterpreted by Darlington (1937) and Matthey (1949) who considered that a multiple system exists in this marsupial. An examination of Agar's drawings shows that, almost certainly, the same type of multiple system is found in *Wallabia* as in *Potorous*, the chromosome number of the former species being the lower by two (Sharman, McIntosh and Barber, 1950). It is probable that a similar mechanism may exist in at least one eutherian mammal, Bovey (1949) having described a trivalent at meiosis in the male of an insectivore, *Sorex araneus*. The number of chromosomes in the female of this species is unknown but Bovey considers that the system is most likely of the $XY_1Y_2 : XX$ type.

2. MATERIAL AND METHODS

The potoroo, *Potorous tridactylus* Kerr, is a common species of rat kangaroo in Tasmania. This study is based on six specimens, four of which (one female and three males) were obtained in the vicinity of Hobart. A fifth specimen was obtained at Branxholm in north-east Tasmania and a sixth at the Arthur Lakes in Central Tasmania.

Various cytological fixatives were tried but acetic alcohol (one part glacial acetic acid to four parts absolute alcohol) and Champy's fixative

TABLE 1

*Chromosome numbers and sex-chromosomes in male marsupials**

DIDELPHIDAE		
Didelphys autrita	20 + XY	Dreyfus & Campos, 1941
D. paraguayensis	20 + XY	Saez, 1931
D. virginiana	20 + XY	Painter, 1922
Lutreolina crassicaudata	20 + XY	Saez, 1938
DASYURIDAE		
Dasyurops maculatus (*Dasyurus maculatus*)	12 + XY	Greenwood, 1923; Koller, 1936
Dasyurus quoll (*D. viverrinus*)	12 + XY	Drummond, 1938
Sarcophilus harrisii (*S. ursinus*)	12 + XY	Greenwood, 1923; Koller, 1936 McIntosh & Sharman, 1952
PERAMELIDAE		
Isoodon obesulus	12 + XY	Drummond, 1933; McIntosh & Sharman, 1952
Perameles gunii	12 + XY	McIntosh & Sharman, 1952
VOMBATIDAE		
Vombatus hirsutus (*Plascolomys mitchellii*)	12 + XY	Altmann & Ellery, 1925
PHASCOLARCTIDAE		
Phascolarctos cinereus	14 + XY	Greenwood, 1923; Koller, 1936
PHALANGERIDAE		
Petaurus breviceps	20 + XY	Drummond, 1933
Schoinobates volans (*Petauroides volans*)	20 + XY	Agar, 1923
Pseudocheirus peregrinus	18 + XY	Altmann & Ellery, 1925; Koller, 1936
Trichosurus vulpecula	18 + XY	" "
MACROPODIDAE		
Bettongia penicillata	26 + XY	Drummond, 1933
B. cuniculus	20 + XY	McIntosh & Sharman, 1952
B. lesueur	20 + XY	Drummond, 1933
Setonyx brachyurus	20 + XY	"
Thylogale billardierii	20 + XY	McIntosh & Sharman, 1952
Megaleia rufa	18 + XY	"
Macropus ocydromus	14 + XY	"
M. tasmaniensis	14 + XY	"
Wallabia elegans (*Macropus parryi*)	14 + XY	Matthey, 1934, 1936
Wallabia rufogrisea	14 + XY	McIntosh & Sharman, 1952
Potorous tridactylus	$10 + XY_1Y_2$	Sharman, McIntosh & Barber, 1950
Wallabia bicolor (*Macropus ualabatus*)	$8 + XY_1Y_2$	Agar, 1923; Sharman, McIntosh & Barber, 1950

* Where names have been changed since the original papers the name under which the cytology of species was described is inserted beneath in brackets. Names are taken from Iredale and Troughton (1934) which appears to be the latest complete nomenclature list.

(chromic-osmic acid) were found most suitable. For metaphase stages of mitosis and meiosis Champy gave good results, but this fixative was found to be entirely unsuitable for prophase stages of meiosis. Excellent fixation of prophase stages, especially diplotene, was obtained with acetic alcohol. The most satisfactory fixation was obtained by cutting out a small portion from the middle of the testis, quickly dropping it in a tube of fixative, and shaking rapidly with the tube corked. Shaking separates the testis tubules from one another thus ensuring quick fixation. Feulgen squashes, made according to the schedules of Darlington and La Cour (1947), were mainly used but some material was micro-

tomed at 15-20 μ and stained by the Feulgen method or with crystal violet. Before making Feulgen squashes the bulk-stained material was examined under the microscope using a magnification of approximately × 400. Sections of tubule undergoing division were selected and separated from the rest of the material prior to squashing. Faded or badly stained Feulgen preparations can be readily restained by the rapid haematoxylin method of Darlington and La Cour (1947).

Figures and microphotos, unless otherwise stated, appear at a magnification of 2,500.

3. MITOSIS AND MEIOSIS

Male and female somatic chromosomes from spermatogonial or ovarian mitoses are illustrated in fig. 1. The male has thirteen chromosomes, the female twelve. In the female six pairs of homologues are easily recognised but in the male three chromosomes are present without

FIG. 1.—Male and female somatic chromosomes. × 2,500.

A. Spermatogonial metaphase; the X chromosome shows differential condensation.

B. Spermatogonial metaphase; the chromosomes show complete somatic pairing with Y_1 left unpaired.

C. Ovarian mitosis from corpus luteum.

equal sized homologues. The largest of these (X) is represented twice in the female (fig. 1C). A smaller rod (Y_2) and a very small chromosome (Y_1) are found in the male only. At metaphase of spermatogonial divisions the X chromosome of the male consists of two differentially staining parts. The short arm and a portion of the long arm near the centromere are undercondensed relative to the remainder of this chromosome and to the autosomes (fig. 1A, B). During the prophase of spermatogonial mitosis this portion of the X chromosome shows precocious condensation. Precocious condensation of sex chromosomes at mitotic prophase has been reported by Crew and Koller (1932) in the mouse. These observations indicate a cycle of condensation of the sex chromosomes (or their heterochromatic portions) differing from that of the autosomes during mitosis. In some mitotic divisions a centromere constriction and minute second arm can be seen on Y_2 (fig. 1B), and a slight constriction, which probably corresponds to the centromere can be seen near one end of Y_1. In some spermatogonial mitoses the chromosomes show complete somatic pairing (fig. 1B) as was the case in the marsupial *Dasyurus* (Koller, 1936).

At pachytene (fig. 4B) the three sex chromosomes of the male have

paired to form a trivalent, consisting at this stage of a deeply stained almost spherical portion connected to a pachytene thread which has the same degree of condensation as the autosomes. The sex elements are thus composed of chromatin of at least two types, one part showing the

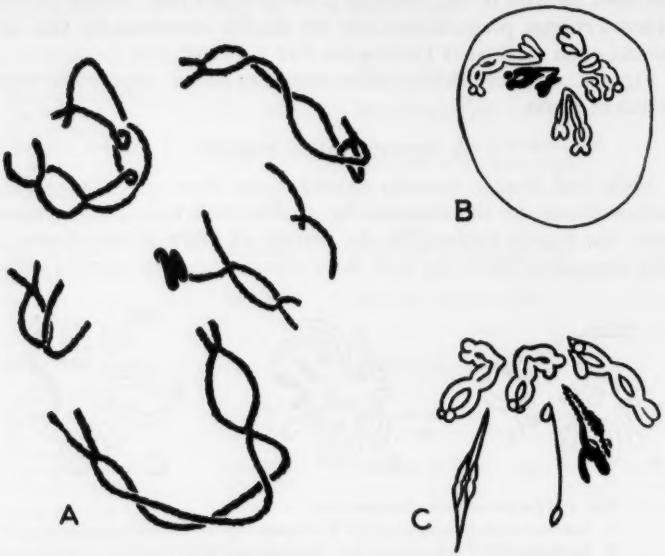

FIG. 2.—Prophase stages of meiosis. × 2,500.

A. Early diplotene; trivalent (at centre) shows condensed and non-condensed portions. One bivalent (top left) broken by squashing.

B. Late diplotene polarisation; trivalent shown in black. (Section.)

C. Early " pre-stretch "; the three longest bivalents are still polarised, the two shortest and the trivalent show pronounced stretch. (Squash.)

early prophase condensation typical of marsupial sex chromosomes, whilst the other part behaves similarly to the autosomes. At early diplotene (fig. 2A) the number of chiasmata per bivalent varies from one (occasionally two) in the shortest bivalent to between three and five in the longest bivalents.

There is no evidence of polarisation at late pachytene or early diplotene. At late diplotene, however, there is a polarised stage at which all the bivalents and the trivalent are attracted to one pole which is apparently determined by the centrosome (fig. 2B). Following polarisation, as the bivalents and trivalent orientate on the spindle, a pronounced " pre-stretch " analogous to that reported by Hughes-Schrader (1943) in mantids occurs. There is a strong repulsion between homologous centromeres and the regions between proximal chiasmata and centromeres are drawn out into long fine threads (figs. 2C, 3A, fig. 5, plate 1). Pre-stretch cannot be confused with anaphase movement since the centromeres are separated by a much greater distance during the stretch

than they are at the onset of first anaphase (fig. 7, plate1). The degree of contraction is also much less than at the beginning of anaphase. Following pre-stretch there is a re-approach of the homologous centromeres probably due to a continuation of the coiling of the chromosomes. No previous reports of this type of chromosome behaviour in marsupials have been made, but our observations (McIntosh and Sharman, 1952) show that it occurs in at least six other species.

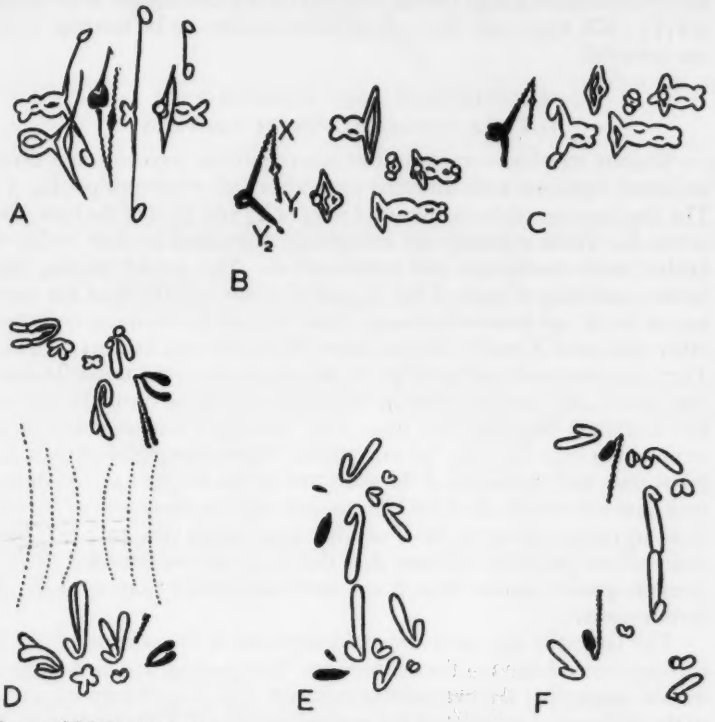

FIG. 3.—Meiosis. × 2,500.

A. " Pre-stretch."

B. First metaphase; one bivalent and the trivalent still slightly stretched; contraction not quite complete.

C. First metaphase; the bivalents show maximum contraction.

D. First anaphase; the heterochromatic portions of the sex chromosomes show marked undercondensation and increased relative length.

E, F. Second anaphase in cells with seven and six chromosomes; X chromosome shows pronounced undercondensation in the heterochromatic arm.

The orientation of the trivalent at metaphase is of the convergent type (Darlington, 1937). It orientates (fig. 3B, C) so that Y_1 and Y_2 point to one pole and X to the other. Thus, the first anaphase (fig. 3D) leads to the production of secondary spermatocytes with six or seven chromosomes, and two types of spermatid are formed after the second

meiotic division (fig. 3E, F). These are a male determining type with five autosomes plus Y_1 and Y_2 and a female determining type with five autosomes plus X.

The cytology of Tasmanian specimens of *Potorous tridactylus* has previously been investigated by Ellery (in Altmann and Ellery, 1925) who reported both male and female numbers to be twelve and sex determination to be of the XY type. By courtesy of Professor O. W. Tiegs we have re-examined Ellery's slides and find the sex mechanism to be of the XY_1Y_2 : XX type, and the male diploid number to be thirteen as in our material.

4. STRUCTURE AND BEHAVIOUR OF THE SEX-CHROMOSOMES IN *POTOROUS*

The sex trivalent consists of two sets of pairing segments and three unpaired segments with different properties and relationships (fig. 5). The first pairing segments consist of most of Y_2 and most of the long arm of the X. These segments are completely autosomal in their cycles of nucleic acid attachment and condensation. The second pairing segments, consisting of most of the Y_1 and of about two-thirds of the short arm of the X, are heterochromatic. They behave in a manner typical of other marsupial X and Y chromosomes (McIntosh and Sharman, 1952). They are overcondensed relative to the autosomes until mid-diplotene (fig. 4B, C) and undercondensed from late diplotene until the end of first anaphase (figs. 4D, E, F, 3D). They are again undercondensed at second anaphase (fig. 3F). At metaphase of spermatogonial mitoses, Y_1 is less than half the length of the short arm of the X (fig. 4A). At diplotene however nearly all of the Y_1 is paired with the short arm of X, and these segments appear to be of nearly equal length (fig. 4C, D). These observations probably indicate that the Y_1 is more contracted during spermatogonial mitoses than is the heterochromatic portion of the X chromosome.

The largest of the three unpaired segments is the portion of the X chromosome containing the centromere. It is a short non-homologous section connecting the two pairing segments (fig. 5), and corresponding to the differential segment of the normal marsupial X chromosome. It is overcondensed during early prophase like the second pairing region (fig. 4B, C) but often becomes undercondensed earlier. The second unpaired segment is short and contains the centromere of Y_2 which appears as a non-staining gap at diplotene (fig. 4C, D). The third unpaired segment is a very small section of the Y_1 chromosome containing the centromere.

Two chiasmata usually occur in the euchromatic segments. The heterochromatic segments invariably show a single chiasma, which can be clearly seen at late diplotene polarisation (fig. 4D, fig. 4, plate 1). Chiasmata have also been observed in the sex-chromosomes of other macropod marsupials and in *Perameles gunnii* (McIntosh and Sharman, 1952). Koller (1936) is of the opinion that " the association of the sex-

FIG. 4.—The structure of the sex chromosomes at mitosis and meiosis. The figures show the condensation of the heterochromatic portions relative to the remainder. × 3,800.

A. Spermatogonial metaphase.

B. Pachytene; heterochromatic portion overcondensed and in the form of a short tight spiral; differential segment of X shows as a short non-homologous region.

C. Early diplotene; heterochromatic portion overcondensed and showing differential and pairing segments.

D. Late diplotene polarisation; heterochromatic portion undercondensed and showing the single chiasma between X and Y_1. Two chiasmata between X and Y_2.

E. " Pre-stretch."

F. First metaphase; maximum contraction of trivalent.

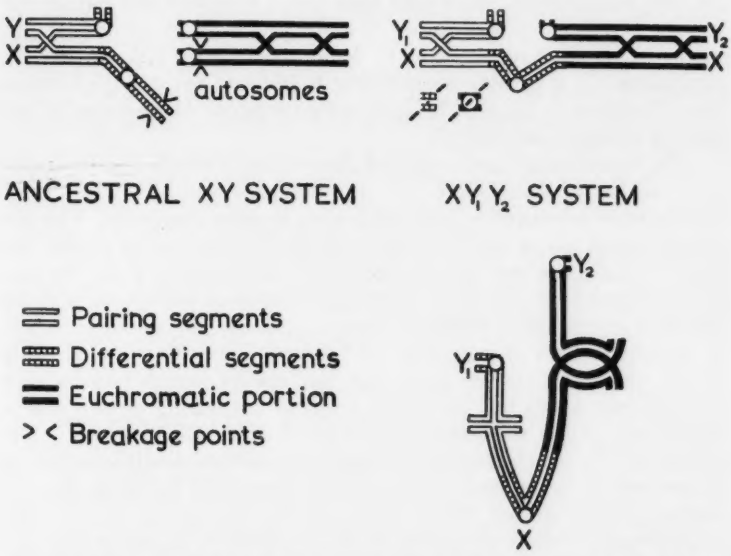

FIG. 5.—The structure of the sex trivalent and its probable method of derivation from an XY system.

chromosomes in the marsupials is without chiasmata and persists as a result of specific attraction based on homology and accentuated by the strong precocious condensation ". As yet we have not examined criti-

cally the sex chromosomes of any representative of the families studied by Koller.

5. THE ORIGIN AND EVOLUTION OF SEX-CHROMOSOME MECHANISMS

A study of sex-chromosomes shows that where the bisexual condition has recently evolved the difference in sex rests on a single gene or a very few genes. The sex chromosomes of such organisms have large euchromatic pairing segments on which free crossing over of many genes is possible, and very small differential segments. Further evolution towards advanced systems such as are found in mammals can take place only if some change, such as inversion, causes the production of differential segments in which crossing over is prevented (Lewis, 1942). The genes on the differential segment of the Y chromosome are never able to undergo crossing over as this chromosome is held permanently in the heterozygous state. They degenerate by mutation or become lost during evolution. The X differential only partly suffers from this disadvantage since it can cross over in the homogametic sex. Genes carried on the X differential segment show complete sex linkage and those carried on the pairing segments partial sex linkage. Both these types of sex linkage are rare in mammals, other than man where complete and partial sex linkage have been demonstrated (Haldane, 1936). Very refined genetic methods are of course necessary to detect partially sex linked genes, so the evidence is at present not sufficient to indicate any definite conclusions (Pontecorvo, 1943). The reasons for the apparent emptiness of the pairing segments are obscure.

The evolutionary path outlined above leads towards genetic inertness and loss of homology of sex chromosomes, a process which may well be more advanced in marsupials than in other mammals. The extremely small size of the Y chromosome in some marsupials studied by Koller (1936), the very strong precocious condensation of the sex elements at prophase, and their undercondensation at metaphase may be regarded as cytological evidence of near inertness. No genetic evidence is available. Loss of homology and genetic inertness coupled with strong precocious condensation may not always permit pairing and crossing over which will inevitably result in loss during meiosis and a breakdown of the sex-determination mechanism. Koller (1936) has shown that the sex-chromosomes of marsupials are at a disadvantage even before meiosis begins since they are more frequently lost from the cell during spermatogonial mitosis than are the autosomes. Koller also found frequent failure of pairing of the sex elements at meiosis and our observations confirm this in other marsupials (Sharman and McIntosh, 1952). No XO sex mechanisms such as are characteristic of some Orthoptera and other groups exist in marsupials yet studied, and indeed their existence has not been proved in any mammal. The most important remaining function of the Y chromosome may be to pair with and ensure segregation of the all important X chromosome at meiosis. In

mammals it is unlikely that difference in sex rests on the presence or absence of a Y chromosome, but on the X chromosome-autosome ratio as was demonstrated by Bridges (1922) in *Drosophila*.

In *Potorous* the structure and behaviour of the elements of the trivalent at mitosis and meiosis show that it has had autosomal material incorporated into it in some way. Y_2 has a nearly terminal centromere and is derived from an autosome, the homologue of which is fused to the ancestral heterochromatic X chromosome. The centromere of the compound X is contained in the heterochromatic portion and the chromosome is monocentric and not dicentric. This means that one centromere has been lost during the process of fusion. The evidence suggests that the trivalent arose by a reciprocal translocation between the ancestral heterochromatic X and the homologue of Y_2, the centromere of which was lost (fig.5). If, as Muller (1940) suggests, a telomere can never become interstitial then a small terminal portion of heterochromatin from the X must also have been lost. Prophase configurations show that the position of the break on the homologue of Y_2 was almost at the centromere, probably in a short heterochromatic region if such exists at this point in marsupial chromosomes. Little genetically active material would then have been lost during the change.

The new XY_1Y_2 system of *Potorous* does not suffer from the disadvantages of the XY systems in which the sex elements are largely inert and the pairing segments small. It has benefited by the incorporation of material showing the same cycle of condensation as the autosomes and has an equal chance with them at division. Loss due to non-disjunction in mitotic divisions is not so likely when the X is permanently attached to an autosome, nor is failure of pairing between X and Y_2 likely at meiosis. Y_1 is no longer necessary to guarantee segregation of the X chromosome at meiosis. If it became genetically inert its loss causing reversion to an XY system might be expected.

The addition of autosomal material to the sex-chromosomes has important implications. Sharman, McIntosh and Barber (1950) have suggested that XY_1Y_2 system of *Wallabia bicolor* was formed in the same way as that of *Potorous*. The multiple system of *Sorex araneus* also contains autosomal material and probably arose in the same way (Bovey, 1949). In all these cases the diploid chromosome number of the female has been reduced by two. Degeneration and disappearance of the small heterochromatic Y_1 chromosome would make the male number equal to that of the female. Presumably the new XY system thus formed would be subject to the same laws which governed the evolution of the old XY system, increasing genetic inertness being coupled with loss of homology. The process of incorporation of autosomal material into the sex mechanism which seems to have occurred in all three species would, if it occurred many times, be responsible for an evolutionary trend towards low chromosome numbers. It is, perhaps, significant that the two marsupials have the lowest chromosome numbers recorded in the mammals. In the Eutheria the average chromosome number is approxi-

mately forty-eight (based on counts of ninety-four species). *Sorex araneus* has twenty-three chromosomes in the male. It is thus possible that this process may be in part, or wholly, responsible for the low chromosome numbers of marsupials (table 1). The XY_1Y_2 system would then be a stage in " the perpetual cycle of disintegration and replacement of the sex-chromosomes " (Darlington, 1939).

6. SUMMARY

Potorous tridactylus has 13 chromosomes in the male, 12 in the female. The male has three sex chromosomes (X_1, Y_1 and Y_2) and the female two Xs. In the X of the male at spermatogonial mitosis, the short arm and a small portion of the long arm are relatively undercondensed.

During meiosis in the male the three sex chromosomes associate and at first division Y_1 and Y_2 pass to one pole and X to the other. The behaviour of the sex chromosomes at mitosis and meiosis shows that they are composed of heterochromatic and euchromatic elements. The short arm of X and the proximal portion of its long arm together with Y_1 show the typical behaviour of marsupial sex chromosomes. Most of the long arm of X and Y_2 behave like the autosomes. The segment of X which pairs with Y_1 is terminal in the short arm and does not include the centromere.

The evolution of sex chromosomes tends towards genetic inertness and loss of homology which may result in non-disjunction at mitosis and failure of pairing at meiosis as has been shown in some marsupials. In *Potorous*, autosomal material has been incorporated in the sex system, probably by reciprocal translocation, and the sex chromosomes are no longer at a disadvantage in division.

It is suggested that the multiple system of *Potorous* may represent a stage in a perpetual cycle of disintegration and replacement of sex chromosomes.

This work was carried out while one author was in receipt of a Commonwealth Research Grant. We wish to thank Mr A. G. Lyne and Mr J. M. de Bavay for their assistance in collecting material, and Professor O. W. Tiegs of Melbourne University for supplying some slides of male and female material used by Ellery (1925). The Fauna Board of Tasmania kindly allowed us to take specimens of this protected animal. We also wish to thank Mr A. J. McIntosh for much help and criticism during the investigation.

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PLATE I.—Squashes of *Potorous tridactylus*. × 2,500.

CH.—Fixation in Champy's fixative. 1 : 3—Fixation in 1 : 3 acetic alcohol. 1 : 4—Fixation in 1 : 4 acetic alcohol. Feul.—stained in Feulgen's leuco-basic fuchsin.

FIG. 1.—Spermatogonial metaphase; Y_2 and X at one o'clock in upper cell. The heterochromatic portion of X is under-condensed (c.f. fig. 1B) Ch., Feul.

FIG. 2.—Metaphase of mitosis from corpus luteum; two X chromosomes at three o'clock (c.f. fig. 1C). 1 : 3, Feul.

FIG. 3.—Diplotene; trivalent at centre. : 14, Feul. restained in haematoxylin.

FIG. 4.—Late diplotene polarisation, considerably displaced by squashing; trivalent at three o'clock showing single chiasma between X and Y_1. 1 : 4, Feul. restained in haemotoxylin.

FIG. 5.—"Pre-stretch"; trivalent at left. 1 : 4, Feul.

FIG. 6.—First metaphase; trivalent at left. (c.f. fig. 3A). The object below the plate is a spermatid. Ch., Feul.

FIG. 7.—Early first anaphase; one bivalent and a X-Y_1 portion of trivalent (at right) have separated. Ch., Feul., restained in haematoxylin.

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POLYPLOID EVOLUTION AND MAMMALIAN CHROMOSOMES

LEO SACHS
John Innes Horticultural Institution
Bayfordbury, Hertford, Herts.

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INTRODUCTION

MAMMALIAN chromosomes are, with some justification, generally considered difficult to study. Adequate techniques are thus of even more importance than with more favourable material. Faulty observations, even within recent years, have often been the direct result of poor techniques. Yet developments in the handling of mammalian chromosomes have lagged far behind the progress made by the smear and squash techniques which are used for plants. Section cutting by the microtome is still the rule for mammals with the osmium chromic mixtures, introduced by Minouchi (Darlington and La Cour, 1947) as the most commonly used fixative. After many years of work on mammalian chromosomes, Matthey (1951*a*) has in fact come to the conclusion that the Minouchi technique—" and only this technique "—gives good results with mammals.

Many species of mammals have a large number of small chromosomes. Apart from any labour saving in the absence of section cutting, squash techniques are thus often necessary for correct observations. The Minouchi type of fixative does not, however, lend itself well to the making of squashes. I have therefore examined a variety of mammals in order to study the possibility of making good squash preparations from the testes. This study has shown that a simple squash technique, far from being inferior to the Minouchi method followed by section cutting, can produce better results.

I have been able to check chromosome numbers in this way and some previously recorded numbers proved to be incorrect. Correct determination has further shown a clear example of polyploid evolution in mammals which had previously been overlooked.

There are thus different reports of chromosome numbers and different reports of techniques. These involve a conflict about facts. On the other hand, the supposed absence of polyploids (Muller, 1940) and the supposed value of " fundamental numbers " (Matthey, 1951*a*) involve a conflict about theory. Application of the correct techniques has given the correct chromosome number. Application of the correct theory has given the correct explanation.

TECHNIQUE

A simple squash method can be used for both mitosis and for

meiosis. Good results with testes were sometimes obtained by fixation in aceto-carmine followed by the normal method of Feulgen staining. But the aceto-carmine did not always produce an adequate fixation. Good results for testes could, however, always be obtained merely by fixation in acetic-alcohol (1 : 3) followed by Feulgen staining and squashing. There was also no difficulty in making the slides permanent.

The condition of the animal is important for obtaining satisfactory preparations. Good results for mitosis can be most easily obtained by using young animals soon after birth. The most suitable age varies with different mammals: it lies in the region of approximately 14 days in those species which have been examined. But the use of young animals is not essential for mitotic figures. Thus in *Mesocricetus auratus*, the golden hamster, comparable results could be seen both in 2 weeks and in 10 weeks old animals. The illustration from the 72 chromosome *Tatera indica ceylonica*, the Ceylon gerbil, was obtained from an animal approximately $5\frac{1}{2}$ months old. (The testis material of the Ceylon gerbil was obtained through the courtesy of Dr W. C. Osman Hill of the London Zoo.)

When investigating mammals which must be caught in the wild, it is often not possible to obtain animals of a desirably young age. The mitotic and meiotic activity of such animals also varies at different seasons of the year. Good results by squashing can, however, still be obtained with animals which can not be kept under laboratory conditions. This was found *e.g.* in *Clethrionomys glareolus*, the bank vole ($2n=56$) and in *Apodemus sylvaticus*, the field mouse, ($2n=48$), which were caught in the grounds of Bayfordbury in January 1952.

This simple squash technique has given uniformly good results in young animals which can be bred in the laboratory, and in older animals which were bred in the laboratory or caught in the wild. La Cour (1944) has shown the results that can be obtained from bone marrow by using a modified type of fixation. It is thus not essential to accept the usual pessimistic outlook on the study of mammalian chromosomes.

THE CHROMOSOMES OF THE HAMSTERS

The hamsters have been divided by Ellerman (1941) on the basis of external animal morphology into *Mesocricetus*, *Cricetus*, *Cricetulus*, and *Phodopus*. There is no data on the chromosomes of *Phodopus*, but the other three groups have now been examined (table 1).

Previous observers had recorded $2n=38$ for *Mesocricetus auratus* (Koller, 1938, 1946; Muldal, 1947) and $2n=14$ for *Cricetulus griseus* (Pontecorvo, 1943; Muldal, 1950). White (1945) cited these counts as an extreme example of widely different chromosome numbers in closely related mammals. Both counts are incorrect.

The correct chromosome number of *Mesocricetus auratus* has been established in young and old males at mitosis and at meiosis. All the golden hamsters in Britain are descended from one original family introduced in 1931. The difference between the previous observations of 38

and the present observation of 44 can thus not be explained by assuming the existence of different chromosome races within the species. Husted *et al.* (1945) had observed two cells with 44 chromosomes in the ovaries of the female golden hamster and Matthey (1951*b*) agrees with this count. Husted had considered that the 44 chromosome cells in the ovary possess a constitution abnormal for the species, but there is no doubt that this is the correct chromosome number of the golden hamster.

The observations of Pontecorvo (1943) on the chromosomes of *Cricetulus griseus*, with its supposed $2n=14$, have also been found incorrect. I was able to obtain some testis material of this species from Dr M. W. Schwentker of Tumblebrook Farm, New York and have established that it has a chromosome number of $2n=22$. I have further found that the allied *Cricetus cricetus* has the same chromosome number of $2n=22$. Matthey (1951*b*) also reports $2n=22$ for *Cricetulus griseus* and *Cricetus cricetus*. There is thus no doubt that $2n=22$ is the correct number for both these species.

The correct chromosome numbers of the hamsters are given in table 1.

TABLE 1

Species	Chromosome number ($2n$)	Number of Mammae
Cricetulus griseus (striped hamster)	22	8
Cricetus cricetus (common hamster)	22	8
Mesocricetus auratus (golden hamster)	44	14-22

THE DEFINITE EXISTENCE OF POLYPLOIDS

The above evidence shows that the hamsters represent a clear example of polyploidy among related species of mammals. *Cricetus* and *Cricetulus* are diploids and *Mesocricetus* is a tetraploid.

The data from external animal morphology also supports the existence of a sudden major evolutionary step in the origin of *Mesocricetus*. This can be seen in the number of mammae in the females (table 1). *Mesocricetus* has 14-22 mammae whereas the other hamsters have characteristically 8 mammae. There is no intermediate forms between the hamsters with 8 mammae and the "multimammate" condition in *Mesocricetus* (Ellerman, 1941). As *Mesocricetus* has arisen by polyploidy one might expect such a sudden major change without intermediate forms.

That *Mesocricetus* is of a later derived origin can further be supported by the geological record (Simpson, 1945).

	Earliest Geological Record		
	Europe		Asia
Diploids Cricetulus Cricetus	Pleistocene Pliocene	← →	Pliocene Recent
Tetraploid Mesocricetus	—	Recent	—

Mesocricetus is only of recent occurrence in Europe and Asia, whereas there are earlier geological records for *Cricetulus* in Europe and Asia and for *Cricetus* in Europe. This absence of earlier geological records for *Mesocricetus* may show that the tetraploid *Mesocricetus* has only arisen from diploids in the recent period.

It has often been observed in plants that related diploids and tetraploids can differ in their geographical distribution. This difference in distribution is also found in the hamsters. *Mesocricetus* shows a more limited geographical distribution than *Cricetus* and *Cricetulus* (Ellerman, 1941 and fig. 1). There are some areas in which the diploids and the tetraploid may co-exist. *Mesocricetus* and *Cricetulus* have been re-

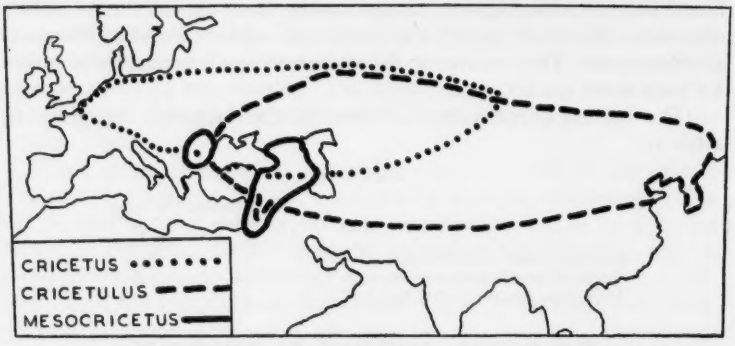

FIG. 1.—Geographical distribution of the Hamsters; *Cricetus* and *Cricetulus*, Diploid; *Mesocricetus*, Tetraploid.

corded in Syria, *Mesocricetus* and *Cricetus* in Roumania, and all three have been recorded in the Caucasus. The origin of the tetraploid can have taken place in or near the Caucasus region.

Data from external animal morphology, the geological record and geographical distribution, thus support the chromosome evidence for polyploid evolution in hamsters.

THE SUPPOSED ABSENCE OF POLYPLOIDS

Polyploidy is now a recognised factor in the evolution of plants. In animals, however, the existence of polyploid evolution is still being disputed. Although it has been shown that polyploid series can be found among parthenogenetic and some hermaphrodite groups of animals, the possible existence of polyploidy is still doubted for bisexual species (White, 1945, 1946). Objections to the possibility of polyploidy among bisexual species has been based both on the theoretically expected sexual conditions in new polyploids, and on the absence of any definite polyploid series among related species. The theoretical objections to the establishment of polyploids (Muller, 1940) have been primarily based, A, on the evidence from triploid intersexes in *Drosophila*, and, B, on the

expected segregation of the sex chromosomes in tetraploids of the heterogametic sex.

A. The sex determining mechanism in *Drosophila* depends on the balance between the number of X chromosomes and the number of autosome sets (Bridges, 1939). Females are produced by an X/A (A = autosome set) of 1·0 and males by an X/A ratio of 0·5. X/A ratios above 1·0 produce super-females, those below 0·5 produce super-males, whereas ratios between 0·5 and 1·0 produce intersexes. Triploids with 2X + 3A, a ratio of 0·67, are thus intersexes. The *Drosophila* data, however, also shows that tetraploids with 4X + 4A, or 2X + 4A, or that triploids with 3X + 3A, still possess the appropriate balance for normal sex determination. The presence of intersexes in the one class of triploids need thus not necessarily be a deterrent to the establishment of polyploids. It is further doubtful how far the *Drosophila* data can be generally applied to all bisexual species. Some animals may have similarities to the type of sex determination found in *Melandrium*. In this type, sex determination is produced by a critical balance dependant on the male determining potencies of the Y chromosome (Warmke, 1946; Westergaard, 1946). Moreover, we do not know how far the sex determining mechanism in mammals, in contrast to *Drosophila*, may be complicated or simplified by the action of hormones. The *Drosophila* data on intersexes is thus not a decisive objection to the establishment of polyploidy among all bisexual species.

B. The second theoretical objection to the establishment of polyploids (Muller, 1940) is the expected segregation of the sex chromosomes in tetraploid individuals of the heterogametic sex. It is assumed that in an XXYY tetraploid, segregation of the X and Y chromosomes at meiosis would produce almost only XY gametes. Any possible mating between an XXXX tetraploid and an XXYY tetraploid would thus tend to produce mainly XXXY individuals. According to the *Drosophila* scheme of sex determination these XXXY individuals would have an abnormal X/A ratio so that they would be unable to reproduce. The XXXX tetraploids would thus be the only properly reproducing animals and the polyploid is then assumed to become effectively homogametic. There is, however, no experimental evidence in animals which has shown this supposed segregation of the sex chromosomes in the tetraploid. The experimental data does show that the establishment of tetraploidy is possible with the *Melandrium* type of sex determination (Warmke and Blakeslee, 1940).

The original production of multivalents by the sex chromosomes is not necessarily a barrier to the establishment of polyploidy. Darlington (1939) has shown how an autosome pair of chromosomes may, in the course of evolution, behave as a sex pair. The opposite process may also take place. A multivalent association of sex chromosomes may thus be changed, during evolution, into one pair of sex chromosomes and one pair of chromosomes which behave like the autosomes. But even if the multivalent sex chromosomes persist, they need not necessarily be

deleterious to survival. Multiple sex chromosomes can still be found in a number of animals (White, 1945). In mammals an XY_1Y_2 sex mechanism has been reported in *Potorous tridactylus*, a rat kangaroo, (Sharman *et al.*, 1950) and *Sorex araneus*, the common shrew, (Bovey, 1949). In both cases, the segregation of the sex trivalents produces a regular distribution of the X and Y chromosomes. There are thus no general theoretical objections to the possible establishment of polyploids.

The actual absence of any definite polyploid series among related species has also been used as evidence against the existence of polyploidy in bisexual species (White, 1946). This objection is no longer valid in view of the present data on the correct chromosome numbers of the hamsters.

The scarcity of reliable data on the chromosomes of other groups, makes it difficult to estimate how far polyploid evolution has taken place in other mammals. In an effort to compare the number of chromosome arms rather than the number of chromosomes, Matthey (1949, 1951*a*) has attempted to reduce all mammalian chromosomes to what he calls " nombre fondamental " (N.F.) or " fundamental number " (F.N.). A metacentric chromosome, with two distinct arms, is counted as an F.N. of 2, and an acrocentric chromosome, with an almost terminal centromere, as an F.N. of 1. There is, however, often no clear-cut difference between some metacentric chromosomes which are given as F.N. of 2, and some acrocentric chromosomes which are given as F.N. of 1. In addition, small metacentric chromosomes are given as F.N. of 2 whereas large acrocentric chromosomes are only given as F.N. of 1. It has often been pointed out that it is not easy to establish the exact position of the centromere in mammalian chromosomes. Indeed, many published drawings of what are called the idiograms of mammalian chromosomes, do not include the location of the centromere. Even with good techniques the exact location of the centromere is sometimes open to doubt. These attempted calculations of "fundamental numbers " in mammals are thus of a doubtful value for estimating the magnitude of different chromosome changes.

Vertebrate and even mammalian evolution is reckoned in millions of years. There have thus been enormous possibilities for structural chromosome changes. Some animal groups may also now possess more than one basic chromosome number, a condition which is known to occur in plants (Darlington and Janaki Ammal, 1945). Changes such as the possible extinction of ancestral form, variations in basic number, and structural chromosome alterations, may now mask the existence of what had originally been produced by polyploidy. That polyploidy definitely does exist can be seen from the data on hamsters. But an evaluation of the general role of polyploidy, and of other possible changes in mammals, is at present mainly prevented by the scarcity of reliable data.

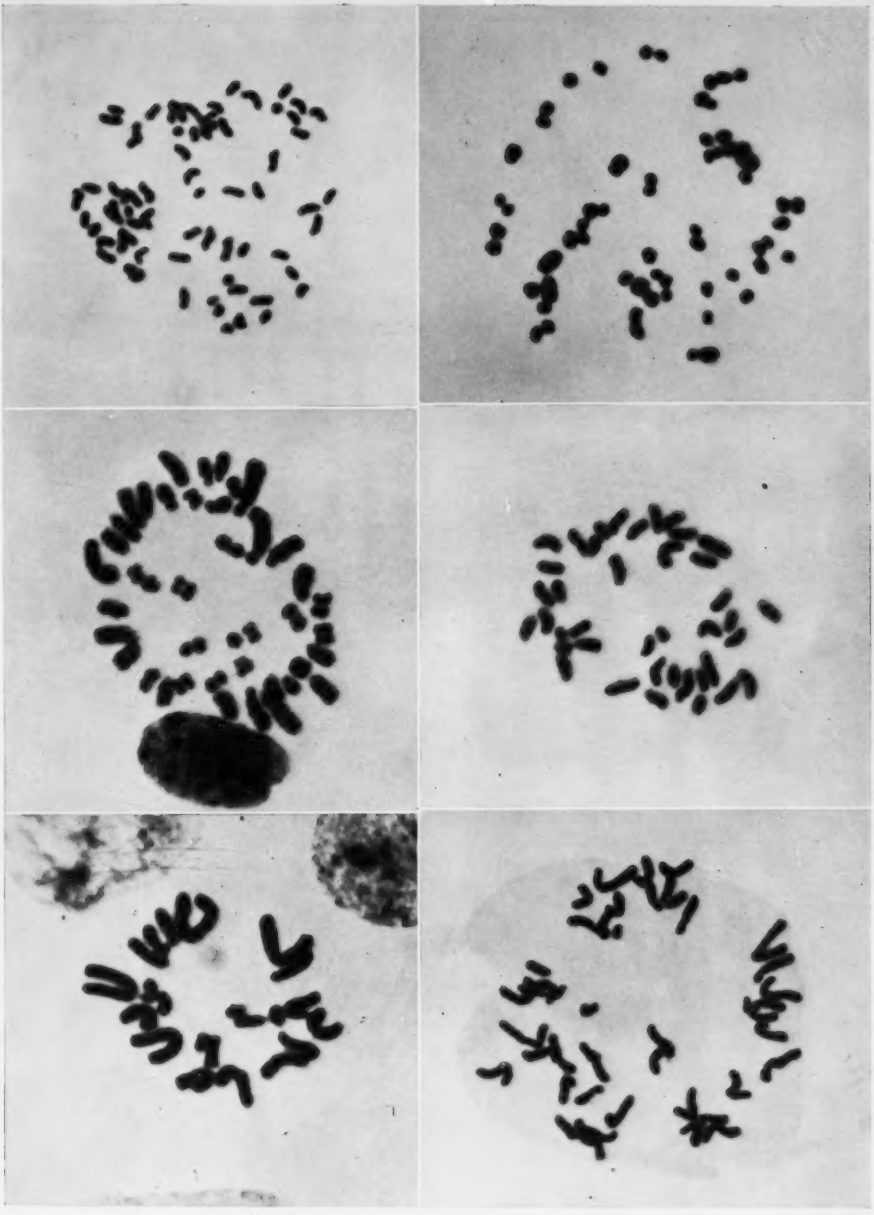

PLATE I.—Microphotographs of spermatogonial cells in mammalian testes.
Feulgen squash × 2,000

FIG. 1.—*Tatera indica ceylonica* (Ceylon gerbil) $2n=72$.
FIG. 2.—*Cavia cobaya* (Guinea pig) $2n=64$.
FIG. 3.—*Rattus norvegicus* (Albino rat) $2n=42$.
FIG. 4.—*Mus musculus* (House mouse) $2n=40$.
FIG. 5.—*Cricetulus griseus* (Striped hamster) $2n=22$.
FIG. 6.—*Mesocricetus auratus* (Golden hamster) $2n=44$.

SUMMARY

1. A simple squash technique has given uniformly good results for mammalian chromosomes.

2. The correct chromosome numbers in the hamsters are $2n=44$ for *Mesocricetus auratus* (the golden hamster), and $2n=22$ for *Cricetulus griseus* (the striped hamster) and *Cricetus cricetus* (the common hamster).

3. The tetraploid *Mesocricetus* has a more limited geographical distribution than the diploids *Cricetulus* and *Cricetus*.

4. The hamsters represent a clear example of polyploid evolution among related species of mammals.

5. Polyploidy is shown to be a definite possibility in the evolution of bisexual species of animals.

Acknowledgment.—I am indebted to Dr C. D. Darlington for suggestions and criticism.

Note added in proof. Since the above was written, Tobias (*S. Afr. Jour. Sci., 48,* 366-373, 1952) has reported $2n=34$ in a South African gerbil *Tatera brantsii draco*. This number is almost half the $2n=72$ which I have found in the Ceylon gerbil *Tatera indica ceylonica*. Polyploid evolution may thus also be found in the mammalian genus *Tatera*.

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THE CHROMOSOMES OF *CHRYSANTHEMUM*, I: THE SPECIES

G. J. DOWRICK
John Innes Horticultural Institution, Bayfordbury, Hertford, Herts.

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1. INTRODUCTION

THIS survey of *Chrysanthemum* species has been carried out in preparation for a study of new forms of the garden *Chrysanthemum*. Previous studies by Japanese workers were concerned with chromosome numbers and with species hybridisation (Shimotomai 1931, 1932, 1937*a* and *b*, 1938; Sugiura, 1937; Takemoto, 1939).

Some 140 species are recognised in the genus, of which 56 have been examined cytologically. There is one basic number throughout ($x=9$) but the numbers found in higher polyploids are no longer exact multiples of the basic number.

2. MATERIALS AND METHODS

The author wishes to thank the following for gifts of seeds or plants: the Directors of the Botanic Gardens at Glasnevin, Edinburgh, Oxford and Lausanne; Royal Botanic Gardens, Kew; The Royal Horticultural Society's Gardens, Wisley; Mr A. P. Balfour of Messrs Sutton, and Professor N. Shimotomai of Hiroshima University, Japan.

Chromosome counts of the species were made from root-tip squashes, using the Feulgen technique with mono-bromo-naphthalene pre-treatment (O'Mara, 1948). Acetic alcohol was used as fixative with hydrolysis for 15 minutes at 60° C; normal time resulted in understaining. A Feulgen Squash technique was used for pollen-mother-cells. Flowers were split up to let in the fixative and, for easier handling, anthers were not removed until after staining. Hydrolysis for 5 to 6 minutes suffices for pollen-mother-cells.

3. SYSTEMATICS AND GEOGRAPHICAL DISTRIBUTION

There are two main centres of distribution; one is in the Mediterranean area, particularly in Algeria and the Canary Islands, the other in China and Japan. The genus has spread throughout most of Europe and Asia. Several species, chiefly perennials, are used as decorative garden plants, the majority of these being of Oriental origin.

Most species have an outer ring of tongue-shaped ray florets surrounding a central mass of disc florets, while in others the ray florets are absent. Conversely horticultural selection has given rise to forms without disc florets. The majority of species are herbs; the rest are semi-shrubs.

Engler and Prantl divide the genus into eight sections, four containing annual and four perennial species. Six of these groups have been studied cytologically. Of the annuals, the sections *Pinardia* and

Coleostephus have been studied. *Ismelia* and *Ammanthus* are still unexamined.

Four sections contain perennial herbs or shrubs. The *Argyranthemum* group, with three-angled fruits, contains 12 to 15 species with yellow or white ray florets. Ten of these originate in the Canary Islands and include *C. frutescens*, the common Marguerite, a semi-shrub with large white flowers often grown for decoration.

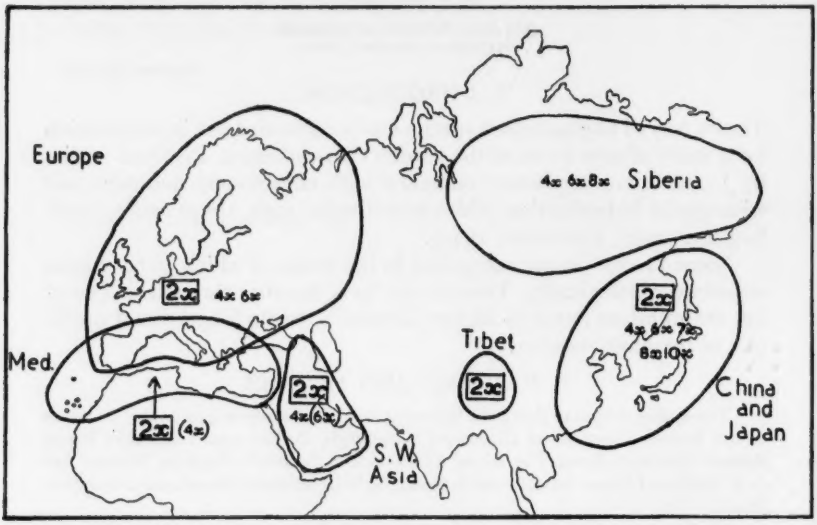


FIG. 1.—World distribution of *Chrysanthemum* species studied in relation to chromosome number. Most of the diploid species occur in the Mediterranean region, where the degree of polyploidy is much lower than in China and Japan. The European species are probably younger than the Oriental.

The three sections with 5 to 10 ribbed fruits contain most species, as follows:

(i) *Pyrethrum:* 50 species with or without long yellow or white ray florets, and with long stalked single capitula giving typical *C. coccineum*, (Pyrethrum) flower heads. The most important is *C. indicum*, long cultivated as a garden plant in China and Japan. Its form is similar to many present day horticultural varieties. There are both "single" and "double" flower heads. This species has been chiefly concerned in the development of present day large-flowering garden Chrysanthemums.

(ii) *Gymnocline:* 14 species having discoid heads with very short yellow or white ray florets. Their centre is in the Caucasus and Dalmatia but a few species are Oriental.

(iii) *Tanacetum:* 50 species with short ray florets or none at all, spread throughout the Northern hemisphere. Seven are N. American, 12 are from the Himalayas and Tibet, while the remainder come from Europe and the Caucasus.

TABLE 1

Geographical Distribution in Relation to Chromosome Number

PYRETHRUM (perennial)

Mediterranean Area

		Chr. Nos.	Species
D		18	*catananche*
D	T	18	*cinerariaefolium* H
D		18	*mawii* H
	S	18	*rotundifolium*
D		18	*serotinum*
D		18	*uliginosum*

S.W. Asia and Caucasus

		Chr. Nos.	Species
	S	18, 36	*alpinum*
D		18, 54	*balsamita*
D		18	*cassium*
D	T	18	*coccineum* H
D		18, (iso)	*millefoliatum*
D		36	*oreades*
D		36	*praealtum*

N. Spain

		Chr. Nos.	Species
D		85, 90, 126, 148, 154, 160 & 171	*maximum* H

Siberia

		Chr. Nos.	Species
	S	36	*ircutianum*
	SH	54	*sibiricum*
D		72	*arcticum*

Europe

		Chr. Nos.	Species
D	S	18, 36	*atratum*
D	S	18, (iso)	
		36	*corymbosum*
D	S	18, 36, 54	*leucanthemum* H
D		18	*parthenium*
	S	54	*ceratophylloides*
D		54	*silvaticum* H
D		198	*lacustre* H

China and Japan

		Chr. Nos.	Species
D		18	*boreale*
	S	18	*lavandulaefolium*
	S	18	*lineare*
	ST	18	*makinoi*
D	ST	18	*nipponicum*
D	S	36, 54	*indicum* H
D		36	*wakasaense* H
D	S	54	*japonense* H
	S	54	*shimotomaii*
	S	54	*weyrichii*
D		63	*rubellum* H
	S	72	*decaisneanum*
	S	72	*ornatum*
D		80	*sonare*
D	S	90	*yezoense*

PINARDIA (annual)

Mediterranean Area

		Chr. Nos.	Species
D	SH	18, 36	*coronarium* H
D	T	18, 36	*segetum* H
D		18	*viscidi-hirtum*

S.W. Asia and Caucasus

	Chr. Nos.	Species
D	18	*segetum* H

Europe

	Chr. Nos.	Species
D	18	*segetum* H

ARGYRANTHEMUM (perennial)

Mediterranean Area

		Chr. Nos.	Species
	S	18	*filifolium*
D	S	27	*frutescens* H

COLEOSTEPHUS (annual)

Mediterranean Area

	Chr. Nos.	Species
D	18	*macrotum*
D	18	*nivellii*
E	18	*myconis*

GYMNOCLINE (perennial)

Europe

		Chr. Nos.	Species
D	S	18	*macrophyllum*

TANACETUM (perennial)

S.W. Asia and Caucasus

	Chr. Nos.	Species
D	18	*argenteum*

China and Japan

		Chr. Nos.	Species
	Su	18	*rupestre*
	S	90	*shimotomaii*

The species are grouped in their respective subgenera and according to geographical distribution. The letters before the chromosome numbers indicate the authors of the counts:

D=Dowrick
S=Shimotomai
SH=Shimotomai and Hara
ST=Shimotomai and Takemoto
SU=Sugiura

The letter H behind the species name indicates it is of horticultural importance.

Fig. 1 shows species distribution in relation to chromosome number. The majority of species, including all annuals, occur throughout the Mediterranean area with most towards the Western end.

Chromosome counts have now been made of 56 species of Chrysanthemum, as shown in table 1. Due to a Botanic Garden peculiarity most of the species collected in the past belong to the sub-section *Pyrethrum* and most of the species studied cytologically belong to this section.

Species situated around the Mediterranean are, with one exception, diploid or have diploid forms. The exception is *C. maximum* ($2n = 90$), found in Northern Spain. The nearby area of the Caucasus and S.W. Asia similarly has only diploids, apart from *C. oreades* and *C. praealtum* which may of course have diploid forms, as yet unexamined.

The other main group, in China and Japan, has a much greater chromosomal range, from diploid to decaploid. It includes *C. indicum* and *C. japonense*, both hexaploid and both possibly involved in the development of garden Chrysanthemums. The degree of polyploidy in relation to geographical distribution is different from that in *Paeonia* (Stern, 1949), where the polyploids are found in the Mediterranean area and only diploids in Asia. Polyploids in Chrysanthemums rise to a much higher multiplication and the chromosomes diminish in size with their multiplication. They are therefore no doubt much older than the *Paeonia* tetraploids.

Polyploidy, in *Chrysanthemum*, is associated with increase in latitude. The diploids occuring mainly near the equator while, as in *Rosa*, only polyploids, *e.g. C. arcticum*, are found in Siberia and the Arctic.

The significant difference in the polyploidy found in the two main centres is no doubt due to difference in age of the species, those in China and Japan being very much older than the Mediterranean species. Only one species, *C. indicum*, has been found in China and Japan in different polyploid forms while in the Mediterranean and S.W. Asia area a number of such species exist suggesting that they are of recent origin and have not, as yet, become sufficiently differentiated to be considered as distinct species.

4. CHROMOSOME NUMBER AND MORPHOLOGY

Chromosome form and size vary little between or within species. In many cases the centromeres are all median or nearly so, as *C. frutescens* (fig. 2).

The species show a polyploid series between 2x—22x types.

Between the species of any one polyploid group there is little difference in chromosome size. Thus in diploid species, the chromosome size ranges from $6 - 8\ \mu$. There is a gradual size diminution through the polyploid series to *C. lacustre* ($2n = 198$) whose average chromosome size is under $3\ \mu$. *C. sonare* ($2n = 80$) has its largest chromosome $4\ \mu$ long, with the majority being about $3{\cdot}5\ \mu$.

FIG. 2.—Somatic chromosome complement of (a) *C. frutescens*, $2n=27$; (b) *C. sonare*, $2n=80$ and (c) *C. lacustre*, $2n=198$. Two small chromosomes with subterminal centromeres are contained in this complement. There is diminution in chromosome size with increase in the degree of polyploidy.

× 1,850

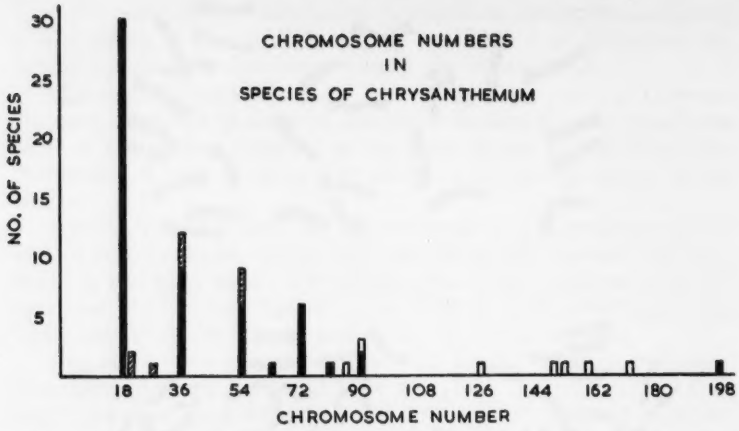


FIG. 3.—Graph of chromosome distribution in species of *Chrysanthemum*. The numbers marked by diagonal lines represent higher polyploid forms of other species. The white numbers between $2n=85$-171 represent counts obtained from different plants of *C. maximum*.

C. maximum originates in the Pyrenees, with a chromosome number of about 90. In garden forms the chromosome number varies from 85 to 171. Counts have been made from 7 different plants and these yielded seven different numbers:

$2n=85$, 90, 126, 148, 154, 160 and 171.

This species, from which Burbank raised the " Shasta Daisy ", has long white ray florets and is grown under such names as " Horace Reed ", " Ester Reed ", etc. It is probable that under cultivation a form with double the chromosome number of the original decaploid was produced, and used for breeding. Crosses between these two extremes of chromosome number would occur and give rise to a series of somatic numbers between 90 and 180.

The plant with 85 has probably arisen from one with 90 chromosomes by loss. The loss does not, in this case, have any great phenotypic effect.

In seven other species, forms are found with different degrees of polyploidy as follows:

$2n=18$, 27 *C. frutescens*	$2n=18$, 54 *C. balsamita*
$2n=18$, 36 *C. atratum* *corymbosum* *segetum*	$2n=36$, 54 *C. indicum* *leucanthemum*

In all these species there are no significant changes in form, apart from size and growth rate, with increase in the degree of polyploidy. They are not considered to be separate species.

5. ACCESSORY CHROMOSOMES

In three species there are atypical chromosomes. *C. lacustre*, $2n = 198$, has a pair of very short euchromatic chromosomes with sub-terminal centromeres, unlike any others found in the genus.

C. corymbosum var. *poteriifolium* and *C. millefoliatum*, two species very similar in vegetative characters, are normally diploid. Plants were found, however, with a small extra chromosome. There are only very slight differences between the somatic chromosomes of these species.

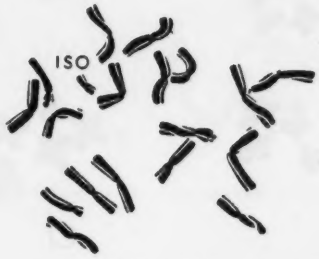


FIG. 4.—The somatic chromosomes of (*a*) *C. corymbosum* var. *poteriifolium*.
× 1,850

The accessory chromosome appears to be identical in form and behaviour. It is an iso-chromosome. It is also heterochromatic, for it is visible in the resting nucleus when stained with the Feulgen technique. Although not in phase with the autosomes in its nucleic acid cycle, it does not lag behind them in its division. Its occurrence among plants was as follows:

	With iso.	Without iso.
C. corymbosum	2	6
C. millefoliatum	1	2

That they are in fact iso-chromosomes with two homologous arms is shown during meiosis. At M I 9 bivalents are formed by the autosomes while the two arms of the accessory chromosome usually pair with each other to form a ring. The accessory chromosomes never pair with the normal set.

Normally the iso-chromosome divides at A I and lags on the metaphase plate during the second division. In one A I separation in *C. millefoliatum*, however, it was seen to misdivide, producing two telocentric chromosomes as in *Secale* and *Campanula*. In *Secale* the standard fragment chromosome of Müntzing (sf) undergoes similar misdivisions giving rise to both small and large telocentric chromosomes. Each of these by secondary misdivision of the centromere can give rise to their corresponding iso-chromosomes. These iso fragments have been seen to misdivide, as in the two species of *Chrysanthemum*. If these supernumeraries survive, no doubt, telocentric chromosomes will arise from them.

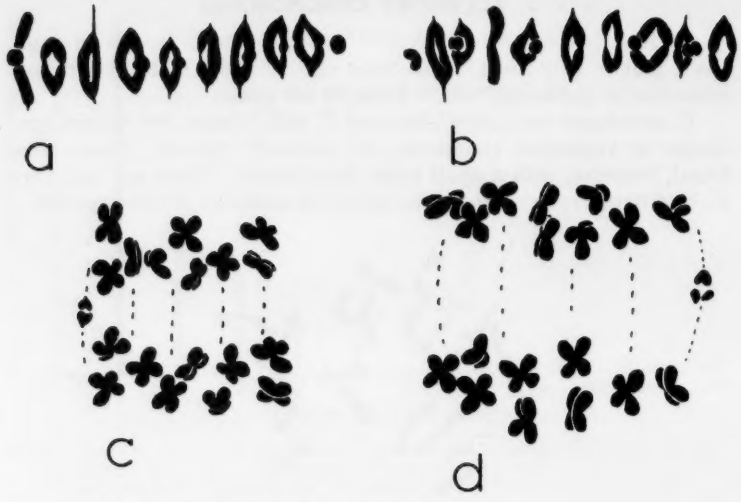


FIG. 5.—*C. millefoliatum.* (*a*) and (*b*) M I; (*c*) and (*d*) A I.
(*c*) shows the normal type of A I found;
(*d*) shows the abnormal division with the formation of two telocentric chromosomes.
× 1,190

The accessory chromosome has little or no effect on the appearance of the plant.

6. MEIOSIS

Most stages of meiosis can be found within the same anther, but two anthers of a flower may be quite out of phase with one another. Anthers of polyploids tend to be larger at meiosis than those of diploids.

(*a*) *Diploid species*

Throughout the genus meiosis is regular except in *C. atratum* (which will be described later). In all diploid species 9 bivalents are formed regularly with an average chiasma frequency of 1·5. The chiasmata are normally terminal (Fig. 6). At M I the centromeres are pulled out towards the poles (plate 1 and fig. 6).

(*b*) *Triploid species*

C. frutescens is the only triploid studied. The number of univalents observed at meiosis varied between 3 and 7. In most divisions a chain of four was found at M I. Associations of more than four chromosomes were not observed, due possibly to the low chiasmata frequency. The univalents divide at either the first or second division and are lost at both divisions.

There are from 0-4 micronuclei in young pollen. Pollen grains vary in size; the majority are haploid, or nearly so, while a few have higher

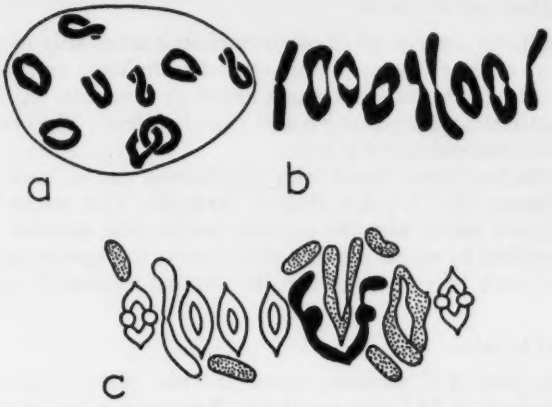


FIG. 6.—(*a*) Diakinesis in *C. macrotum*, $2n=18$ with interlocking bivalents; (*b*) M I in *C. coronarium*, $2n=18$; (*c*) M I in *C. frutescens*, $2n=27$.

(*a*) × 900
(*b*) and (*c*) × 2,000

numbers. Fig. 7 shows the radii of mature grains and the four peaks are, no doubt, due to grains with x, 2x, 3x, and 6x chromosomes. The species exists only in its triploid form in this country. From its meiotic behaviour it appears to be autotriploid. The slightly smaller form found in the Canary Islands is fertile and probably diploid.

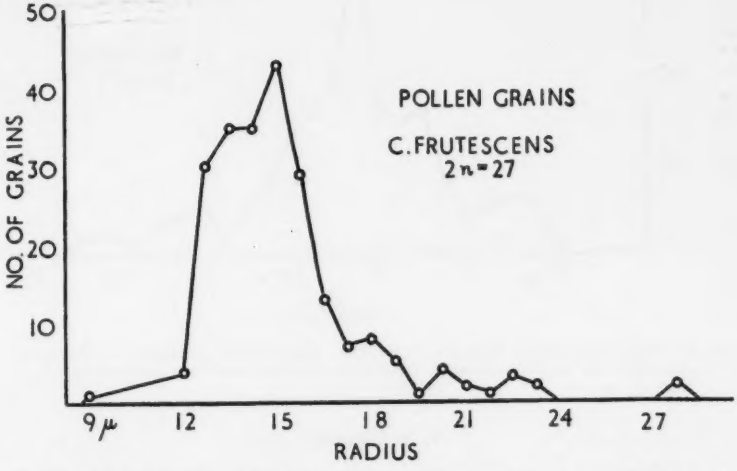


FIG. 7.—Graph of pollen grain size in *C. frutescens*, $2n=27$. There is an absence of grains with lower chromosome numbers; they become eliminated more readily than the more diploid grains.

(*c*) *Tetraploids and hexaploids*

In tetraploid and hexaploid species meiosis is necessarily more complex though even here some species behave like diploids. In *C. silvaticum* ($2n=54$) M I plates usually contain either 27 bivalents, 25 bivalents and 1 quadrivalent or 23 bivalents and 2 quadrivalents. The occurrence of trivalents and univalents is rare.

A I cells have been found with 36 chromosomes at each pole in *C. leucanthemum* the " Ox-Eye Daisy " ($2n=36$). This results in diad formation and pollen with the somatic chromosome number. It is a possible method by which the hexaploid form of this species has arisen, a process which may be related to the unusual meiosis of *C. atratum*.

(*d*) *Higher polyploids*

In one plant of *C. maximum*, where $2n=160$ (plate 1) there are 10 to 30 univalents at M I; the remaining chromosomes are in groups of 2, 3, 4, 5 and 6. Bridges are seen at A I when some univalents divide. The cytoplasm is small compared with the nucleus and hence there is little elimination of univalents at either first or second division. Mature pollen grains therefore show little variation in size, much less than in the triploid *C. frutescens* (fig. 8).

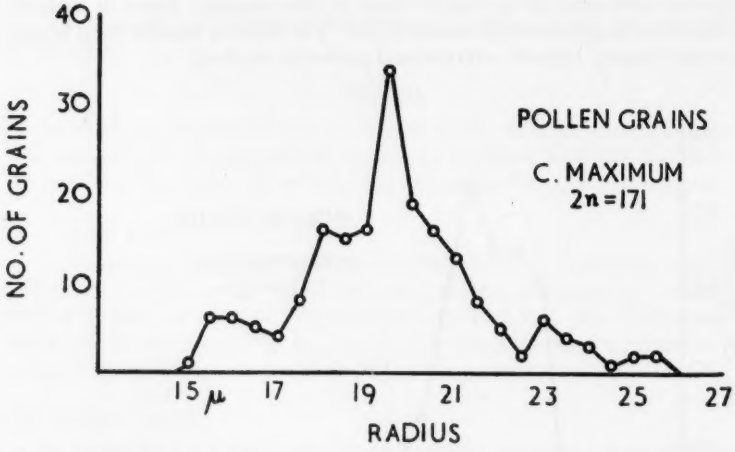
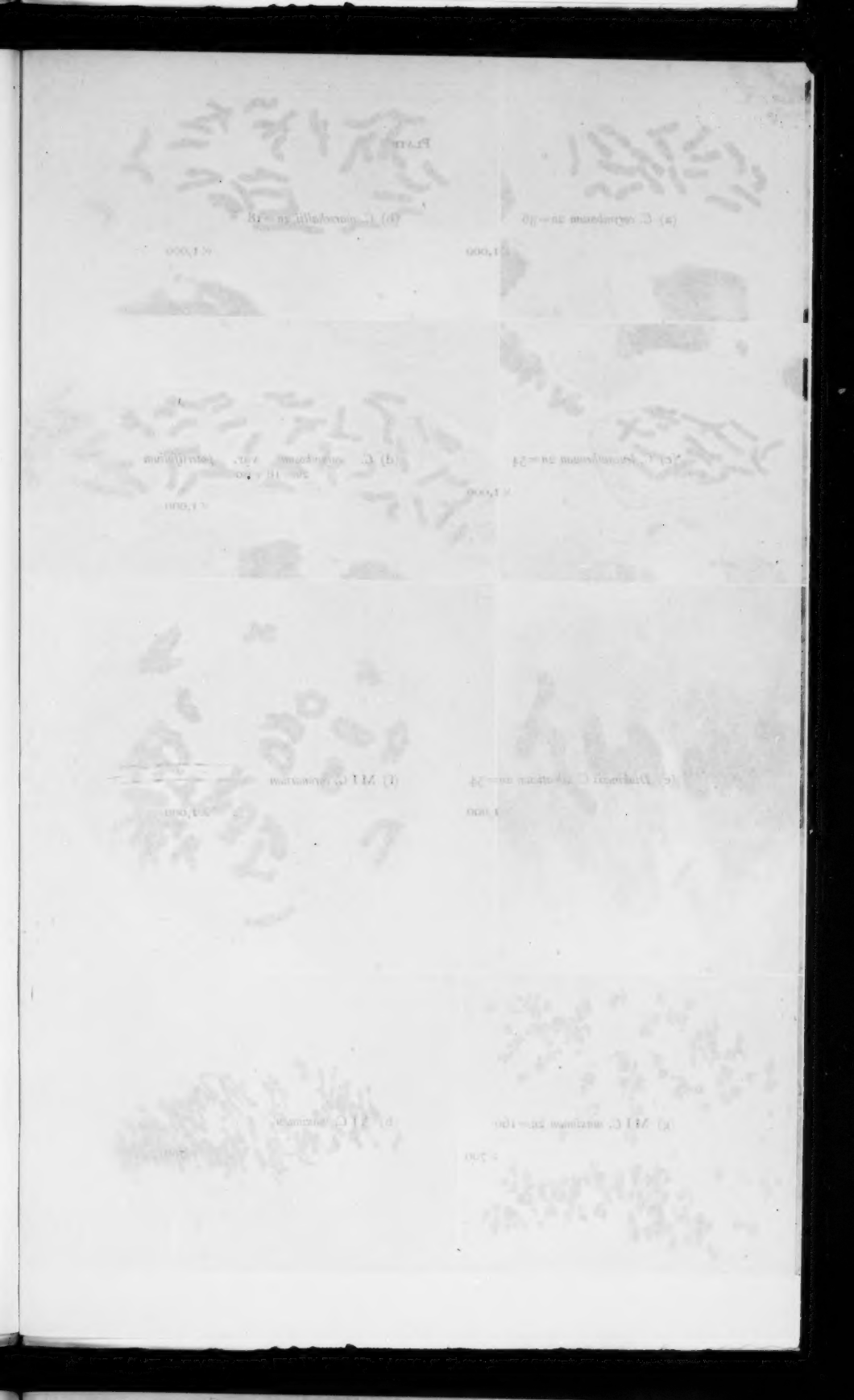


FIG. 8.—Pollen grain size in *C. maximum*, $2n=160$. There is little variation in pollen grain size where the large chromosome number is concerned and is much less than in *C. frutescens*, $2n=27$.

7. SUMMARY

1. Of the 140 species in Chrysanthemum 56 (in 6 of the 8 sections) have now been examined cytologically. The basic number throughout is 9 (list p. 000). The chromosomes differ little amongst themselves.



Plate

(a) *C. corymbosum* $2n=36$

× 1,000

(b) *C. marschallii* $2n=18$

× 1,000

(c) *C. leucanthemum* $2n=54$

× 1,000

(d) *C. corymbosum* var. *poteriifolium* $2n=18+\text{iso}$

× 1,000

(e) *Diakinesis C. silvaticum* $2n=54$

× 1,000

(f) M I *C. coronarium*

× 1,000

(g) M I *C. maximum* $2n=160$

× 700

(h) A I *C. maximum*

× 700

2. There are two main centres of diversity in the genus, one in China and Japan, and the other at the Western end of the Mediterranean. Most Mediterranean species are annuals and diploid, the Oriental ones chiefly perennial and polyploid. Arctic species are entirely polyploid.

3. Eight species show varying degrees of polyploidy. In one of these, *C. frutescens*, only the sterile triploid form has been examined. *C. maximum* varies, in its garden forms, between 85 and 171 chromosomes.

4. Increase in chromosome number in the polyploid series is accompanied by decrease in chromosome size.

5. Accessory heterochromatic iso-chromosomes occur in two species. These undergo misdivision and give telocentrics.

6. Meiosis is regular in the botanic garden species studied except *C. atratum*. There is little loss of unpaired chromosomes in the higher polyploids owing to the small size of the pollen mother cell relative to the nucleus.

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GENETICS OF COLD TOLERANCE IN MAIZE AND SWEET CORN SEED

GORDON HASKELL
John Innes Horticultural Institution, Bayfordbury, Hertford, Herts.

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THERE are several important factors influencing the adaptation and establishment of sweet corn as a horticultural crop in England and other European countries. As conditions in England at sowing times are colder and wetter than those for which varieties have been selected under American conditions (Mather and Haskell, 1949), ability of seeds to germinate in moist or wet soil at low temperatures is very important. A recent preliminary survey of the germination of hybrid dent corn from Iowa and Minnesota grown at Bayfordbury made it clear that use of American strains, unselected for specific English conditions, was also limited by poor germination. This has renewed interest in the problem of maize germination at low temperatures.

There have been numerous studies on cold testing of maize under unfavourable conditions to determine the factors preventing germination. Wernham (1951) has reviewed these investigations in detail. Only genetical aspects will here be considered, based on results from a study of reciprocal crosses between inbred sweet and dent maize. The aim is to evaluate maternal influence, endosperm and pericarp effects and the role of hybrid vigour.

Literature Review. Reciprocal differences are known. Smith (1935) found that an inbred maize resistant to *Gibberella zeae* crossed with a susceptible gave F_1 hybrids as resistant as the resistant parental inbred; there was strong indication that (susceptible × resistant) was more susceptible than (resistant × susceptible). Pinnell (1949) also found strong maternal influence on seedling emergence in cold tests of reciprocal crosses between inbred dent lines. Mortimore (in Wernham, 1951) showed significant differences in germination between reciprocals of high and low germinating inbreds: the maternal parent was more important in determining differences. Rush (in Wernham, 1951) considered embryo constitution unimportant because embryos in reciprocal crosses are identical. He suggested that pericarp and/or endosperm are important.

Inbreds may differ from their hybrids in cold tolerance. Thus Haskell and Singleton (1949) believed that heterosis usually shows its effects in cold tests of inbred and hybrid dent maize by improved germination of the hybrids. They interpreted a reverse instance as due to negative heterosis. On the other hand, Rossman (1949) considered

that freezing tolerance of the seed is not a manifestation of hybrid vigour, maternal seed characteristics being more important than embryo constitution.

EXPERIMENTAL METHODS

The seeds investigated were from hand pollinations of inbred strains grown at New Haven, Conn., in 1947, viability still being satisfactory. The material included Conn 520, a starchy dent (*Su*) maize inbred; four sweet corn (*su*) inbreds, viz. C4, C40, T51 and C95; reciprocal hybrids between the sweets and dent, and a few sweet hybrids. The pedigrees are given in table 1.

TABLE 1

Germination of Sweet Corn Inbreds and a Dent Maize Inbred and their Reciprocal Hybrids after Treatment for 10 days at 50° F. (Based on 50 seeds per box except those marked †.)

No.	Pedigree No.	Parentage	Inbred or Hybrid	Genotype Endosperm	% Germination		
					Plot 1	Plot 2	Mean
1	859 selfed	Conn 520	I	Su/Su/Su	90	96	*93·0*
2	820 selfed	C 4	I	su/su/su	48	74	*61·0*
3	859 × 820	Conn 520 × C 4	H	Su/Su/su	88	96	*92·0*
4	859 × 807	Conn 520 × C 4	H	Su/Su/su	52	90	*71·0*
5	807 × 859	C 4 × Conn 520	H	su/su/Su	84†	96†	*90·0*
6	830 selfed	C 40	I	su/su/su	46	74	*60·0*
7	859 × 830	Conn 520 × C 40	H	Su/Su/su	80	100	*90·0*
8	830 × 859	C 40 × Conn 520	H	su/su/Su	48	92	*70·0*
9	850 selfed	T51	I	su/su/su	45†	60†	*52·5*
10	859 × 850	Conn 520 × T51	H	Su/Su/su	100	96	*98·0*
11	850 × 859	T51 × Conn 520	H	su/su/Su	16	76	*46·0*
12	869 selfed	C95	I	su/su/su	0	5	*2·5*
13	859 × 869	Conn 520 × C95	H	Su/Su/su	76	94	*85·0*
14	869 × 859	C95 × Conn 520	H	su/su/Su	40	84†	*62·0*
15	850 × 830	T51 × C40	H	su/su/su	8	24	*16·0*
16	830 × 850	C40 × T51	H	su/su/su	12	60	*36·0*
17	820 × 830	C4 × C40	H	su/su/su	90†	100†	*95·0*

Two lots of fifty seeds of each were sown in seed boxes on 3rd April 1951. Unsterilised field soil, on which maize had previously grown, was used. The boxes were placed at random in two plots in the controlled temperature room. The soil was evenly watered each day to keep it moist. Seed boxes were transferred to a warm greenhouse after 10 days at 50° F., and records made of germinations (Haskell, 1949). Final percentage germinations were calculated and then transformed to angles for the analysis of variance. These data are also given in table 1. The wet weights of seedlings were also determined on 1st May, *i.e.* 18 days after transference to warmth.

EFFECTS OF TREATMENT

(i) *Differences in Cold Tolerance.* The analysis of variance given in table 2 shows that the strains genuinely differ in ability to germinate following low temperature treatment. There is also a highly significant difference between plots; this could partly be accounted for by overhead illumination in the controlled temperature room being nearer to

one plot. This might have created a local rise of temperature, but more likely it promoted convection currents, thus drying out soil faster

TABLE 2

Analysis of Variance of Cold Tolerance Data

(% Germinations transformed to angles)

Stem	S. S.	N	M.S.	V.R.	t	P
Lines	14,552·51	16	*909·53*	13·65	—	>0·001
Inbreds v. Hybrids	1,487·20	1	*1487·20*	—	4·73	>0·001
Su-su Reciprocals	957·90	1	*957·90*	—	3·79	0·01 – 0·001
Remainder	12,107·41	14	*864·81*	12·99	—	>0·001
Plots	2,523·50	1	*2523·50*	—	6·15	>0·001
Line-Plots (Error)	1,065·46	16	*66·59*			
Total	18,141·47	33				

in the seed boxes adjacent to the lights. These boxes would then have better germinations, as their soil would be drier and pathogens would have less opportunity of acting on the seeds. The use of means of the two plots in later calculations reduces error due to plot differences.

There are significant differences in cold tolerance between inbreds and all their hybrids, when the item for the numerous lines is broken down into its components. Thus hybrid vigour is important. The mean square for reciprocal dent-sweet crosses is less than that for hybrid vigour but is also highly significant. Therefore in addition to hybrid vigour effects, the way a cross is made between inbred sweet corn and starchy dent maize influences the cold tolerance of seeds.

(ii) *Reciprocal Differences.* The germination of four pairs of reciprocal crosses between dent maize and sweet corn are given in table 3. The endosperm in maize is triploid, *i.e.* there are three chromosome sets in each of its cells. Two chromosome sets are provided by the seed parent

TABLE 3

Effect of Reciprocal Crosses between Sweet Corn and Dent Maize on Cold Tolerance of Seeds

Cross \ Endosperm	% Germination Su/Su/su	Su/su/su	Difference
Conn 520-C4	71·0	90·0	*– 19·0*
,, ,, -C40	90·0	70·0	*+ 20·0*
,, ,, -T51	98·0	46·0	*+ 52·0*
,, ,, -C95	85·0	62·0	*+ 23·0*
Mean	86·0	67·0	

but one dose only comes from the pollen parent. Hence endosperms may differ genetically in reciprocal crosses according to the dominance of the genes involved. The *Su* gene is dominant irrespective of dosage; seeds containing it are always starchy. Thus all seeds in the reciprocal crosses, even those on sweet corn ears, had starchy endosperms. Hybrid seeds taken from dent ears, *i.e.* with endosperms containing two *Su* doses, were considerably more tolerant of cold than hybrid seeds from sweet ears which had only one *Su* dose in their endosperms. There was one exception. This was Conn 520 crossed with C4; yet Haskell (1949) has already shown that C4 can be more cold tolerant than Conn 520 under more prolonged treatment. Although Conn 520 has somewhat

higher germination than C4 in the present experiment, this exception proves valuable when considering the influence of the pericarp.

The wet weights of seedlings in reciprocal crosses are given in table 4. Maternal effects would have been in evidence, as readings were taken

TABLE 4

Seedling Weights of Reciprocal Crosses

Cross \ Endosperm	Weight Su/Su/su	Su/su/su	Difference
C.520-C4	1·57	1·28	+0·29
„ -C40	1·13	0·74	+0·39
„ -T51	1·20	1·39	−0·19
„ -C95	0·64	0·76	−0·12
Mean	1·14	1·04	

(Figures refer to mean weight in gms of seedlings weighed after 18 days in a warm greenhouse)

only eighteen days after transference to warmth. There is no indication, however, that seedlings from *Su/Su/su* endosperms are necessarily heavier than those from *Su/su/su* endosperms. Nor is there evidence in any one direction of undue influence of cytoplasmic effects from the embryo itself.

It thus seems that although there are reciprocal differences in cold tolerance of maize seeds, neither cytoplasmic influences nor dosage of the *Su* gene are likely to be of primary importance; other factors must be acting.

(iii) *Heterosis.* The analysis of variance (table 2) shows that inbreds and hybrids differ significantly in germination. The means of inbred parents can therefore be compared with means of their reciprocal F_1's to estimate more easily the effects of hybrid vigour. These data are given in table 5. Here it is seen that most F_1's have higher germinations than their parental averages. The cross of Conn 520 with T51

TABLE 5

Influence of Hybrid Vigour on Maize Cold Tolerance

Endosperm	Cross	% Germination Inbred Parental Means	Reciprocal F_1 Means	Difference
Starchy	Conn 520-C4	77·0	80·5	+3·5
	„ „ -C40	76·5	80·0	+3·5
	„ „ -T51	72·8	72·0	−0·8
	„ „ -C95	47·8	73·5	+25·7
Sugary	C40-T51	56·3	26·0	−30·3
	C4-C40	60·5	95·0	+34·5
	Mean	65·2	71·2	

shows no increase, while the F_1 is considerably lower in the sweet hybrid of C40 with T51. This might be an example of negative heterosis. Thus it may be concluded that hybrid vigour usually, but not always, promotes better cold tolerance.

The comparative germinations of inbreds and hybrids under field

conditions in England are given in fig. 1. These graphs are based upon the germination of 80 hybrid and 40 inbred American sweet corns, not necessarily related to each other. They were sown on 13th May 1948

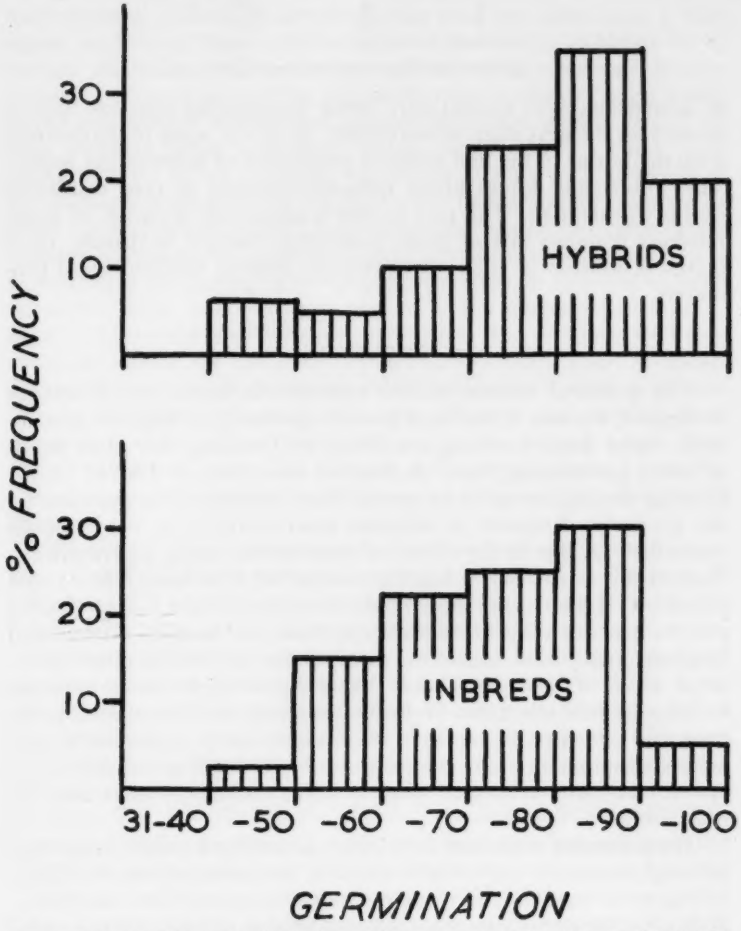

FIG. 1.—Percentage frequency distributions of the germinations of 40 inbred and 80 hybrid sweet corns grown at Merton, Surrey on 13th May 1948.

at Merton, Surrey. Range of germination is similar in both types but there are comparatively more hybrids with higher germinations.

The effects of gradual inbreeding are given in table 6. Open-pollinated varieties of sweet corn which had been self-pollinated for one or more generations are grouped according to their stage of inbreeding. It is readily seen that germination falls off with advances

TABLE 6

Influence of Inbreeding on Germination of Sweet Corn under Early Field Conditions in England

Generation of Inbreeding	No. of lines	Mean % Germination
1	34	36·6
2	16	15·1
3 or more	35	10·9

(40 seeds per row sown 20th April 1951)

in inbreeding, even though only better germinating lines are used as parents for the next stage of inbreeding. However, some of the decrease is no doubt due in the first stages to production of homozygous lethals. Thus inbreeding brings about reduced tolerance to cold conditions during germination. This may be due to actual loss of vigour, or to the resulting homozygosity of genes controlling reaction to disease, or to failure of inbreds to adapt themselves to difficult environmental conditions.

DISCUSSION

The genetical control of cold tolerance in sweet corn is already recognised, because it has been possible gradually to improve germination under English sowing conditions by breeding only from plants in better germinating lines. A diagram was given by Haskell (1948) showing the improvement in germination resulting from selection for this property. Response to selection must therefore be polygenically controlled, *i.e.* due to the effects of many genes acting quantitatively. Thus in this experiment it was not unexpected that lines differ in cold tolerance. Haber (1938) has already found that inbred strains of sweet corn in America differ in resistance to heat and drought. Heyne and Brunson (1940) have attributed susceptibility to heat to direct pleiotropic action of the *su* gene: hence the same gene might also be expected to influence cold tolerance. Hybrid vigour, maternal cytoplasmic influences and pericarp differences of seeds might also be important in promoting germination at low temperatures besides endosperm differences due to the *Su-su* relation. These possible influences must now be examined.

From crossing sweet and dent corns, Kiesselbach (1926) found that although sometimes seed weights decrease, they usually increase. There is thus not always an effect due to change in endosperm constitution. Hybrid vigour shows mostly as increased weights of seed, embryo, endosperm and perhaps pericarp, varying according to the cross. Embryos tend to increase somewhat more than endosperms, but pericarps increase far less, if at all, probably because they are maternal tissue and only indirectly influenced by the pollen parent. (Sweet × dent) give considerably higher heterotic effect than (dent × sweet), which show no change in seed weight. Yet (dent × sweet) seeds generally have better tolerance in the present experiment. Hence the improved germination of F_1 seeds cannot wholly be due to heterosis.

Besides heterotic effects, change in endosperm composition can itself cause marked increase in seed weight when sweets and dents are hybridised. However, pericarp weight is unaffected in sweet corn crosses which show heterosis, and only very slightly in sweet-dent crosses. The main physical differences between sweet corn and dent seeds is that sugary seeds have higher moisture content than dents, as shown by F_1 crosses. According to Kiesselbach (1948) they differ chemically mainly in having more water-soluble polysaccharides. Johann (1935) maintained that disease resistance of germinating maize seeds is bound up with chemical rather than with anatomical differences. These, even with regard to hilum openings, would not account for differences of susceptibility to *Diplodia* in the field. Yet better quality commercial sweet corns characteristically have reasonably tender pericarps: plant breeders have doubtless selected for thinner pericarps. Hence if thinner pericarps permit pathogens to enter more readily, or enzymes to leach out faster, sweet corn seeds will tend to be less tolerant than those of dents. This would have helped to give rise to the prevailing view that sweet corn seeds are not so hardy as those of other kinds of maize. Segregations of genetically similar starchy and sugary seeds *on the same ears* should therefore have similar cold tolerance and this has been observed by Haskell and Singleton (1949). Such seeds would have similar tolerances, regardless of their endosperms' genetical constitution, because the pericarps are all of maternal constitution, dent in this instance.

The above experiment supports previous work on hybrid vigour. One of the genetical aspects influencing tolerance of maize seeds to cold wet soil is whether they are heterozygous or homozygous. Maize is an outbreeding species carrying genes for disease resistance or susceptibility in a heterozygous condition. Inbreeding unmasks these genes releasing highly susceptible lines, yet at the same time permitting selection of more resistant lines. So-called homozygous, uniform, inbred lines usually have some seedlings killed off but not others. This may be due partly to differences in soil and other environmental conditions, as emphasised by Pinnell (1949), and partly to cryptic variation within the lines. This is masked to the ordinary eye, but manifests itself when the strains are faced with environmental conditions which select out the individuals genetically more adaptable to them.

In open-pollinated maize populations the paired chromosomes carry most of their genes in a heterozygous state. The cytoplasm has thus become adapted to this internal relation expected in an out-breeding species. Hence the reaction of the same cytoplasm to homozygous chromosomes found in inbreds might in effect reduce the general viability. This would establish a state of physiological unbalance in the cellular metabolism. Hence unfavourable environmental conditions react more readily on homozygous inbred lines than on balanced open-pollinated varieties or heterozygotes, *i.e.* F_1 hybrids where the state of unbalance has been restored. This has already been observed in other

quantitative characters of maize, such as variation in height and silking dates (Haskell, 1953). That inbreeding is itself injurious is shown by the gradual reduction in germinations with progressive stages of inbreeding. Major recessive lethal genes are rapidly eliminated at the first or second stage, but these would still not sufficiently account for continual decrease with continued inbreeding.

Inbreds are therefore less likely to germinate as well as hybrids under poor conditions, even when lethals have been removed. This has to be taken into account when introducing a new American hybrid into England after suitable testing. Its inbred parents may not be so adaptable and may germinate only with difficulty under the same sowing conditions. Hence it might sometimes be feasible to grow inbreds, both for stock and hybrid seed, in more suitable parts of the Commonwealth. This would not, however, alter the suitability of the hybrid to English sowing conditions, provided that the seed is correctly harvested, dried and stored.

SUMMARY

1. Germinations of inbred sweet corns, dent maize, and their reciprocal hybrids were studied following treatment in soil kept for 10 days at 50° F. (10° C.). Strains differ in cold tolerances. Inbreds generally have lower germinations than hybrids, attributed to physiological consequences of inbreeding.

2. Hybrids between sugary (*su*) and starchy (*Su*) corn germinate better from a starchy mother plant. This is probably due to the excessive permeability of sweet corn pericarp which has been selected for tenderness.

3. Hybrids with improved earliness and corresponding cold tolerance could be introduced in England although their inbred line parents might have to be raised abroad.

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SOME GENOTYPIC FREQUENCIES AND VARIANCE COMPONENTS OCCURRING IN BIOMETRICAL GENETICS

J. A. NELDER
National Vegetable Research Station, Wellesbourne, Warwicks.

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1. INTRODUCTION

MATHER (1948, 1949) has developed an analysis of the variation of characters which are phenotypically continuous in expression, using data derived from the crossing of two true-breeding lines. In essence the model used makes the following assumptions: the genes are additive in effect; the segregations are Mendelian; no selection operates during the experiment; and the distribution of residuals (or non-heritable components) is independent of the genotype. In the usual notation, genotypes **AA**, **Aa**, **aa** of a locus a produce on the character in question average effects d_a, h_a, and $-d_a$ respectively, the zero point on the scale being the mid-parent value. The recombination fraction between loci a and b is written p_{ab}. With this model, it is found that the expectations of certain mean squares, such as the variance of F_2 plants, the variance of F_3 family means, etc., can be expressed as known linear functions of three quantities D, H, E, where D is a function of the d's and the p's only, H is a function of the h's and the p's only, and E is an environmental variance dependent in structure on the layout of the experiment. Mather has shown that when linkage is absent, D and H are the same throughout and are equal to Σd^2 and Σh^2 respectively, but that when linkage is present, D takes the form $\Sigma d^2 + \Sigma' f(p_{ab}) d_a d_b$, where Σ denotes summation over all the loci concerned, Σ' summation over all pairs of loci and $f(p)$ is a function of the recombination fractions which changes with the generations of, say, selfing or backcrossing; H is similarly modified. Mather (1949) gives expressions for the coefficients of D and H and the form of $f(p)$ for selfing up to F_4 and for backcrossing for two generations. In this paper these expressions will be generalised to cover any generation of selfing or backcrossing to either parent and, in obtaining these expressions, the effect of k generations of selfing or backcrossing on the relative proportions of genotypes of two linked genes will also be derived.

2. METHODS

The genetical structure of a population with regard to two linked genes is defined by ten quantities, four giving the proportions of the homozygotes **AABB**, **AAbb**, **aaBB**, and **aabb**, four the proportions of the single heterozygotes, **AABb**, **aaBb**, **AaBB**, and **Aabb**, and two

giving the proportions of the two phases, coupling and repulsion, of the double heterozygote **AaBb**. These ten quantities will be written as a column vector $\mathbf{x}=\{x_1, x_2, \cdots, x_{10}\}$ with components representing the genotypes as in table 1.

TABLE 1

	AA	Aa	aa
BB	1	2	3
Bb	4	5C/6R	7
bb	8	9	10

Thus, assuming Mendelian segregation with recombination fraction p throughout, {0, 0, 0, 0, 1, 0, 0, 0, 0, 0} represents the F_1 of a cross between **AABB** and **aaBB** and $\left\{\frac{q^2}{4}, \frac{pq}{2}, \frac{p^2}{4}, \frac{pq}{2}, \frac{q^2}{2}, \frac{p^2}{2}, \frac{pq}{2}, \frac{p^2}{4}, \frac{pq}{2}, \frac{q^2}{4}\right\}$ the F_2 from such an F_1, where $q=1-p$. In considering the descendants of two true-breeding lines, five quantities suffice to describe the population since $x_1=x_{10}$, $x_3=x_8$, and $x_2=x_4=x_7=x_9$ throughout. The ten components will be retained here for the sake of generality.

The effect on a population of selfing or of crossing to an independent population is to replace the components of $\mathbf{x}$ by other components linearly dependent on them. Writing $\mathbf{x}_0$ for the original population and $\mathbf{x}_1$ for the population after one generation of, say, selfing we can put

$$\mathbf{x}_1=\mathbf{T}\mathbf{x}_0$$

where $\mathbf{T}$ is a 10×10 matrix, the *transition matrix* of selfing, and the components of $\mathbf{T}$ depend on p but not on $\mathbf{x}_0$. The effect of k generations of selfing is to produce proportions $\mathbf{x}_k$ given by

$$\mathbf{x}_k=\mathbf{T}^k\mathbf{x}_0$$

The problem of finding these k-th generation proportions is thus reduced to the problem of finding the k-th power of a matrix. This is most conveniently done by using the so-called spectral matrices of $\mathbf{T}$. It can be shown that, to each latent root of $\mathbf{T}$, λ_α say, there corresponds a matrix $\mathbf{Q}_\alpha$ such that

$$\text{(i)}\quad \mathbf{T}=\Sigma\lambda_\alpha\mathbf{Q}_\alpha$$

$$\text{(ii)}\quad \mathbf{Q}_\alpha^2=\mathbf{Q}_\alpha$$

$$\text{(iii)}\quad \mathbf{Q}_\alpha\mathbf{Q}_\beta=\mathbf{O},\ \alpha\neq\beta$$

It follows immediately that $\mathbf{T}^k=\Sigma\lambda_\alpha^k\mathbf{Q}_\alpha$, so that $\mathbf{T}^k$ is expressed as a linear function of the spectral matrices $\mathbf{Q}$ with coefficients equal to the k-th power of the latent roots.

It should be noted that the method fails for biparental progeny because the components of $\mathbf{x}_1$ are then quadratic functions of the components of $\mathbf{x}_0$ instead of linear ones, so that a method based on linear transformations becomes inapplicable.

3. THE TRANSITION MATRIX FOR SELFING

By considering the progeny derived from selfing the various genotypes, we find as the transition matrix for selfing

$$\mathbf{T}=\begin{pmatrix}
1 & \frac{1}{4} & 0 & \frac{1}{4} & \frac{q^2}{4} & \frac{p^2}{4} & 0 & 0 & 0 & 0\\
0 & \frac{1}{2} & 0 & 0 & \frac{pq}{2} & \frac{pq}{2} & 0 & 0 & 0 & 0\\
0 & \frac{1}{4} & 1 & 0 & \frac{p^2}{4} & \frac{q^2}{4} & \frac{1}{4} & 0 & 0 & 0\\
0 & 0 & 0 & \frac{1}{2} & \frac{pq}{2} & \frac{pq}{2} & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & \frac{q^2}{2} & \frac{p^2}{2} & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & \frac{p^2}{2} & \frac{q^2}{2} & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & \frac{pq}{2} & \frac{pq}{2} & \frac{1}{2} & 0 & 0 & 0\\
0 & 0 & 0 & \frac{1}{4} & \frac{p^2}{4} & \frac{q^2}{4} & 0 & 1 & \frac{1}{4} & 0\\
0 & 0 & 0 & 0 & \frac{pq}{2} & \frac{pq}{2} & 0 & 0 & \frac{1}{2} & 0\\
0 & 0 & 0 & 0 & \frac{q^2}{4} & \frac{p^2}{4} & \frac{1}{4} & 0 & \frac{1}{4} & 1
\end{pmatrix}$$

Column 4, for example, expresses the fact that selfing genotype 4 (**AABb** from table 1) produces $\frac{1}{4}$ of genotype 1, *i.e.* **AABB**, $\frac{1}{2}$ of genotype 4, *i.e.* **AABb**, and $\frac{1}{4}$ of genotype 8, *i.e.* **AAbb**.

Since $|\mathbf{T}-\lambda\mathbf{I}|=(1-\lambda)^4(\frac{1}{2}-\lambda)^4\begin{vmatrix}\frac{q^2}{2}-\lambda & \frac{p^2}{2}\\ \frac{p^2}{2} & \frac{q^2}{2}-\lambda\end{vmatrix}$

we have, for the latent roots of $\mathbf{T}$, 1 fourfold, $\frac{1}{2}$ fourfold, $\frac{1}{2}(1-2p)$, and $\frac{1}{4}(1-2pq)$. The spectral matrix for $\lambda=1$ is given by

$$\mathbf{Q}_1=\begin{pmatrix}
1 & \frac{1}{2} & 0 & \frac{1}{2} & \frac{1}{2(1+2p)} & \frac{p}{1+2p} & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & \frac{1}{2} & 1 & 0 & \frac{p}{1+2p} & \frac{1}{2(1+2p)} & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & \frac{1}{2} & \frac{p}{1+2p} & \frac{1}{2(1+2p)} & 0 & 1 & \frac{1}{2} & 0\\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & \frac{1}{2(1+2p)} & \frac{p}{1+2p} & \frac{1}{2} & 0 & \frac{1}{2} & 1
\end{pmatrix}$$

for $\lambda=\frac{1}{2}$ by

$$\mathbf{Q}_3=\begin{pmatrix}
0 & -\frac{1}{2} & 0 & -\frac{1}{2} & -\frac{1}{2} & -\frac{1}{2} & 0 & 0 & 0 & 0\\
0 & 1 & 0 & 0 & \frac{1}{2} & \frac{1}{2} & 0 & 0 & 0 & 0\\
0 & -\frac{1}{2} & 0 & 0 & -\frac{1}{2} & -\frac{1}{2} & -\frac{1}{2} & 0 & 0 & 0\\
0 & 0 & 0 & 1 & \frac{1}{2} & \frac{1}{2} & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & \frac{1}{2} & \frac{1}{2} & 1 & 0 & 0 & 0\\
0 & 0 & 0 & -\frac{1}{2} & -\frac{1}{2} & -\frac{1}{2} & 0 & 0 & -\frac{1}{2} & 0\\
0 & 0 & 0 & 0 & \frac{1}{2} & \frac{1}{2} & 0 & 0 & 1 & 0\\
0 & 0 & 0 & 0 & -\frac{1}{2} & -\frac{1}{2} & -\frac{1}{2} & 0 & -\frac{1}{2} & 0
\end{pmatrix}$$

for $\lambda=\frac{1}{2}(1-2p)$ by

$$\mathbf{Q}_3=\begin{pmatrix}
0 & 0 & 0 & 0 & -\frac{1-2p}{4(1+2p)} & \frac{1-2p}{4(1+2p)} & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & \frac{1-2p}{4(1+2p)} & -\frac{1-2p}{4(1+2p)} & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & \frac{1}{2} & -\frac{1}{2} & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & -\frac{1}{2} & \frac{1}{2} & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & \frac{1-2p}{4(1+2p)} & -\frac{1-2p}{4(1+2p)} & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & -\frac{1-2p}{4(1+2p)} & \frac{1-2p}{4(1+2p)} & 0 & 0 & 0 & 0
\end{pmatrix}$$

and for $\lambda=\frac{1}{2}(1-2pq)$ by

$$\mathbf{Q}_4=\begin{pmatrix}
0 & 0 & 0 & 0 & \frac{1}{4} & \frac{1}{4} & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & -\frac{1}{2} & -\frac{1}{2} & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & \frac{1}{4} & \frac{1}{4} & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & -\frac{1}{2} & -\frac{1}{2} & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & \frac{1}{2} & \frac{1}{2} & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & \frac{1}{2} & \frac{1}{2} & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & -\frac{1}{2} & -\frac{1}{2} & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & \frac{1}{4} & \frac{1}{4} & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & -\frac{1}{2} & -\frac{1}{2} & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & \frac{1}{4} & \frac{1}{4} & 0 & 0 & 0 & 0
\end{pmatrix}$$

The transition matrix for k generations of selfing is given by

$$\mathbf{T}^k=\mathbf{Q}_1+(\tfrac{1}{2})^k\mathbf{Q}_2+\left(\frac{1-2p}{2}\right)^k\mathbf{Q}_3+\left(\frac{1-2pq}{2}\right)^k\mathbf{Q}_4.$$

It may be noted in passing that as $k\to\infty$, $\mathbf{T}^k\to\mathbf{Q}_1$.

By putting $\mathbf{x}_1=\{0, 0, 0, 0, 1, 0, 0, 0, 0, 0\}$ and evaluating $\mathbf{x}_k=\mathbf{T}^{k-1}\mathbf{x}_1$ we have the genotype frequencies for the F_k generation from two true-breeding lines for two genes in coupling. In fact,

$$\mathbf{x}_k = \begin{pmatrix} \frac{1}{2(1+2p)} - \frac{1}{2^k} - \frac{(1-2p)^k}{2^{k+1}(1+2p)} + \frac{(1-2pq)^{k-1}}{2^{k+1}} \\ \frac{1}{2^k} - \frac{(1-2pq)^{k-1}}{2^k} \\ \frac{p}{1+2p} - \frac{1}{2^k} + \frac{(1-2p)^k}{2^{k+1}(1+2p)} + \frac{(1-2pq)^{k-1}}{2^{k+1}} \\ \frac{1}{2^k} - \frac{(1-2pq)^{k-1}}{2^k} \\ \frac{(1-2p)^{k-1}}{2^k} + \frac{(1-2pq)^{k-1}}{2^k} \\ \frac{-(1-2p)^{k-1}}{2^k} + \frac{(1-2pq)^{k-1}}{2^k} \\ x_{k7} = x_{k4} \\ x_{k8} = x_{k3} \\ x_{k9} = x_{k2} \\ x_{k10} = x_{k1} \end{pmatrix}$$

To obtain the corresponding frequencies for two genes in repulsion, it is only necessary to interchange the 1st and 3rd components, the 8th and 10th components, and the 5th and 6th components. The limiting frequencies as $k \to \infty$ for the coupling phase are given by $\mathbf{Q}_1 \mathbf{x}_1$, that is the proportions of **AABB** and **aabb** are $\frac{1}{2(1+2p)}$, those of **AAbb**, **aaBB**, $\frac{p}{1+2p}$, and the remainder are zero.

4. THE BACKCROSS TRANSITION MATRIX

By considering the result of crossing each genotype to **AABB**, we find for the transition matrix of backcrossing to **AABB**

$$\mathbf{T} = \begin{pmatrix} 1 & \frac{1}{2} & 0 & \frac{1}{2} & \frac{1}{2}q & \frac{1}{2}p & 0 & 0 & 0 & 0 \\ 0 & \frac{1}{2} & 1 & 0 & \frac{1}{2}p & \frac{1}{2}q & \frac{1}{2} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & \frac{1}{2} & \frac{1}{2}p & \frac{1}{2}q & 0 & 1 & \frac{1}{2} & 0 \\ 0 & 0 & 0 & 0 & \frac{1}{2}q & \frac{1}{2}p & \frac{1}{2} & 0 & \frac{1}{2} & 1 \\ \multicolumn{10}{c}{\textit{Remaining five rows all zeros}} \end{pmatrix}$$

The transition matrices for backcrossing to the other homozygotes may be obtained by an interchange of appropriate columns in the above matrix. The characteristic roots are 0 sixfold, $\frac{1}{2}$ twofold, 1, and $\frac{1}{2}q$.

The spectral matrix for $\lambda = 1$ is given by

$$\mathbf{Q}_1 = \begin{pmatrix} 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ \multicolumn{10}{c}{\textit{Remainder zeros}} \end{pmatrix}$$

for $\lambda = \frac{1}{2}$ by

$$\mathbf{Q}_2 = \begin{pmatrix} 0 & -1 & -2 & -1 & -2 & -2 & -3 & -2 & -3 & -4 \\ 0 & 1 & 2 & 0 & 1 & 1 & 2 & 0 & 1 & 2 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 1 & 1 & 1 & 2 & 2 & 2 \\ \multicolumn{10}{c}{\textit{Remainder zeros}} \end{pmatrix}$$

and for $\lambda = \frac{1}{2}q$ by

$$\mathbf{Q}_3 = \begin{pmatrix} 0 & 0 & 0 & 0 & 1 & \frac{p}{q} & \frac{1}{q} & 0 & \frac{1}{q} & \frac{2}{q} \\ 0 & 0 & 0 & 0 & -1 & -\frac{p}{q} & -\frac{1}{q} & 0 & -\frac{1}{q} & -\frac{2}{q} \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & -1 & -\frac{p}{q} & -\frac{1}{q} & 0 & -\frac{1}{q} & -\frac{2}{q} \\ 0 & 0 & 0 & 0 & 1 & \frac{p}{q} & \frac{1}{q} & 0 & \frac{1}{q} & \frac{2}{q} \\ & & & & & \textit{Remainder zeros} & & & & \end{pmatrix}$$

so that $\mathbf{T}^k = \mathbf{Q}_1 + (\frac{1}{2})^k \mathbf{Q}_2 + (\frac{q}{2})^k \mathbf{Q}_3$.

To obtain the frequencies resulting from the k-th backcross of an F_1 in coupling to parent **AABB** we put $\mathbf{x}_0 = \{0, 0, 0, 0, 1, 0, 0, 0, 0, 0\}$ and evaluate $\mathbf{x}_k = \mathbf{T}^k \mathbf{x}_0 =$

$$\begin{pmatrix} 1 - \frac{1}{2^{k-1}} + \left(\frac{1-p}{2}\right)^k \\ \frac{1}{2^k} - \left(\frac{1-p}{2}\right)^k \\ 0 \\ \frac{1}{2^k} - \left(\frac{1-p}{2}\right)^k \\ \left(\frac{1-p}{2}\right)^k \\ \textit{Remainder zeros} \end{pmatrix}$$

The frequencies for the backcross to the other parent, **aabb**, are obtained by interchanging x_1 and x_{10}, x_2 and x_9, and x_4 and x_7 in the above expression.

5. APPLICATION TO VARIANCE COMPONENTS

(I) *Selfing Series*

Consider the F_k generation following the crossing of two true-breeding lines. The plants in this generation can be divided into families of parents in the F_{k-1}; the parents can be divided into families of plants in the F_{k-2}, and so on. We shall say that plants of F_k which had a common ancestor in F_l but not in F_{l+1} form an *l-group*. We define σ^2_{lFk} as the component of variance of $(k-2-1)$-group means within $(k-2)$-groups in the total heritable variance of the F_k, with obvious modifications when l takes the extreme values 1 and $k-1$. To fix the ideas, consider the F_4 and write x_{ijk} for the measurement of the k-th plant having grandparent i and parent j. Suppose i runs from 1 to r, j runs from 1 to s, and k runs from 1 to t. The total sum of squares $\sum_{ijk}(x_{ijk} - x\ldots)^2$ (where a dot suffix denotes, as usual, an averaging over the suffix concerned) can be split up into three parts, namely

$$\sum_{ijk}(x_{ijk} - x\ldots)^2 = \sum_{ijk}(x_{ijk} - x_{ij\cdot})^2 + t\sum_{ij}(x_{ij\cdot} - x_{i\cdot\cdot})^2 + st\sum_{i}(x_{i\cdot\cdot} - x\ldots)^2$$

corresponding to the primary analysis of variance in a double split-plot experiment. For the corresponding mean squares, we write

$$V_{3F4}=\frac{1}{rs(t-1)}\sum_{ijk}(x_{ijk}-x_{ij\cdot})^2$$

$$V_{2F4}=\frac{1}{r(s-1)}\sum_{ij}(x_{ij\cdot}-x_{i\cdot\cdot})^2$$

$$V_{1F4}=\frac{1}{r-1}\sum_{i}(x_{i\cdot\cdot\cdot}-x_{\cdot\cdot\cdot\cdot})^2$$

then

$$\mathcal{E}(V_{3F4})=\sigma^2{}_{3F4}+E$$

$$\mathcal{E}(V_{2F4})=\sigma^2{}_{2F4}+\frac{\sigma^2{}_{3F4}}{t}+E$$

$$\mathcal{E}(V_{1F4})=\sigma^2{}_{1F4}+\frac{\sigma^2{}_{2F4}}{s}+\frac{\sigma^2{}_{3F4}}{st}+E$$

where $\mathcal{E}$ denotes expectation and E is the non-heritable component of variance. The E occurring in the three expressions above is not necessarily the same in each case. In the notation of Mather (1949), $V_{3F4}=\overline{V}_{F4}$, $V_{2F4}=\overline{V}_{\overline{F4F}}$ and $V_{1F4}=V_{\overline{F4G}}$.

The form of $\sigma^2{}_{lFk}$ in the presence of linkage has been given by Mather (1949) as far as the F_4 components. We shall now derive the general expression for $\sigma^2{}_{lFk}$.

The means of any generation of plants are unaffected by linkage. In particular, a gene occurring in the homozygous state contributes $\pm d$ to the mean in the k-th generation of selfing independently of k, while a gene occurring in the heterozygous state contributes $\frac{h}{2^k}$ to the same mean. Now σ^2_{lFk} can be expressed as the difference of the total variance of $(k-l-1)$-group means and $(k-l)$-group means in F_k, ignoring the variation within means. In computing the variance of $(k-l)$-group means, ignoring the variation within means, the variate is that of the mean in the $(k-l)$-th generation of selfs while the frequencies will be those of the F_l. These are shown in table 2.

Evaluating the variance of the means in table 2, and subtracting it from the variance obtained by substituting $l+1$ for l, we find, after some algebraic reduction, that

$$\sigma^2_{lFk}=\frac{1}{2^l}[d_a^2+d_b^2+2d_ad_b(1-2p)^l]+\frac{1}{2^{2k-l-1}}[h_a^2+h_b^2+2h_ah_b(1-2pq)^l(1-4pq)]$$

For linkage in repulsion, the term in d_ad_b has its sign changed.

Noting that $1-4pq=(1-2p)^2$ and $1-2pq=1-2p+2p^2$, it will be found that these values for σ_{lFk} are in agreement with those up to σ^2_{3F4} given by Mather (1949).

(ii) *Backcross Series*

In the progeny of the k-th backcross to one of the parents, the components of variance may be defined in an exactly analogous manner

TABLE 2

Genotype	Mean of $(k-l)$-th generation of selfs derived from genotype	Frequency in F_l
AABB	d_a+d_b	as previously derived for F_k with l written for k
AaBB	$\frac{h_a}{2^{k-l}}+d_b$	
aaBB	$-d_a+d_b$	
AABb	$d_a+\frac{h_b}{2^{k-l}}$	
AaBb.C.	$\frac{h_a}{2^{k-l}}+\frac{h_b}{2^{k-l}}$	
AaBb.R.	$\frac{h_a}{2^{k-l}}+\frac{h_b}{2^{k-l}}$	
aaBb	$-d_a+\frac{h_b}{2^{k-l}}$	
AAbb	d_a-d_b	
Aabb	$\frac{h_a}{2^{k-l}}-d_b$	
aabb	$-d_a-d_b$	

to those of F_k. It will be found, however, that these components of variance contain cross-terms in $d\,h$. These can be eliminated if an average variance over the two k-th backcrosses is taken. We shall denote this average variance by σ^2_{lBk} and a similar argument to that in the previous section shows that

$$\sigma^2_{lBk}=\frac{1}{2^{2k-l}}[d_a^2+d_b^2+2d_ad_b(1-p)^l(1-2p)+h_a^2+h_b^2+2h_ah_b(1-p)^l(1-2p)]$$

As before, the sign of the d_ad_b term must be changed for linkage in repulsion. In Mather's notation

$$\sigma^2{}_{1B1}=\tfrac{1}{2}\mathcal{E}(V_{B1}+V_{B2})$$
$$\sigma^2{}_{2B2}=\tfrac{1}{2}\mathcal{E}(V_{B11}+V_{B22})$$
$$\sigma^2{}_{1B2}+\frac{1}{n}\sigma^2{}_{2B2}=\tfrac{1}{2}\mathcal{E}(V_{\overline{B11}}+V_{\overline{B22}})$$

where n is the number of plants per family in the second backcross.

6. SUMMARY

General formulae are given for the relation between the initial genotype frequencies of two linked genes in any population and those obtaining after k generations of selfing, or of backcrossing to one of the homozygotes.

These formulae are used to derive expressions for certain variance components occurring in biometrical genetics.

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A LETHAL ALLELE OF DILUTE IN THE HOUSE MOUSE

A. G. SEARLE
University College, London, W.C.1.

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THE recessive gene "dilute lethal" (symbol d^l) first occurred in the pure line C 57 BL/Gr in June 1950, as a spontaneous mutation. It was noticed that three out of a litter of seven had a dilute fur-colour. Later they developed neurological defects and died soon after weaning. The parents were perfectly normal in behaviour and fur-colour.

DESCRIPTION

On a C 57 Black background the mutants can first be distinguished at the age of three or four days by their lighter pigmentation. For the first nine days or so they behave normally and start walking. But soon they tend to overbalance. If at rest they lean over more and more to one side or the other until they fall, with an associated hyperextension of the limbs on the opposite side. On righting themselves, nine-day mutants may show slight convulsive limb movements. When walking they topple over without warning and the hind-limbs tend to drag.

At twelve days, the mutant is hardly able to walk at all, but can move more easily when on its side, often going in circles in apparent efforts to right itself, which take much longer than normal. Convulsions are more marked, being clonic in nature with the fore-limbs rapidly beating up and down.

By fifteen or sixteen days the whole syndrome is well-developed. A fit usually begins whenever the animal is disturbed or when it overbalances and struggles to right itself, but sometimes the cause is uncertain. Following loss of balance, the convulsive seizures start as a rule when an upright posture is regained. Opisthotonus soon sets in, with an arching upwards of head and tail to such an extent that they often meet each other. The forepaws are characteristically brought up on each side of the nose. Then the animal often overbalances; violent righting movements are made and another fit may be precipitated.

Later, when the symptoms become more severe, the hind-limbs become increasingly involved and the mutant makes violent bouncing movements. Limb convulsions associated with the opisthotonus may become tetanic, with the animal staying rigid for a second or two. When upright, it can at best make weak lurching movements, although it is more active when on its side. Sometimes it is found lying on its back in the cage and it will eat in this position. If food and water are placed within reach it can eat and drink normally. Hyperacuity is

sometimes shown, but on other occasions the same animal shows no reaction to a loud noise.

The plate shows characteristic postures adopted by mutants during and after convulsive fits. When an affected mouse is in the nest it can subdue its limb convulsions somewhat by clutching on to straws, etc.;

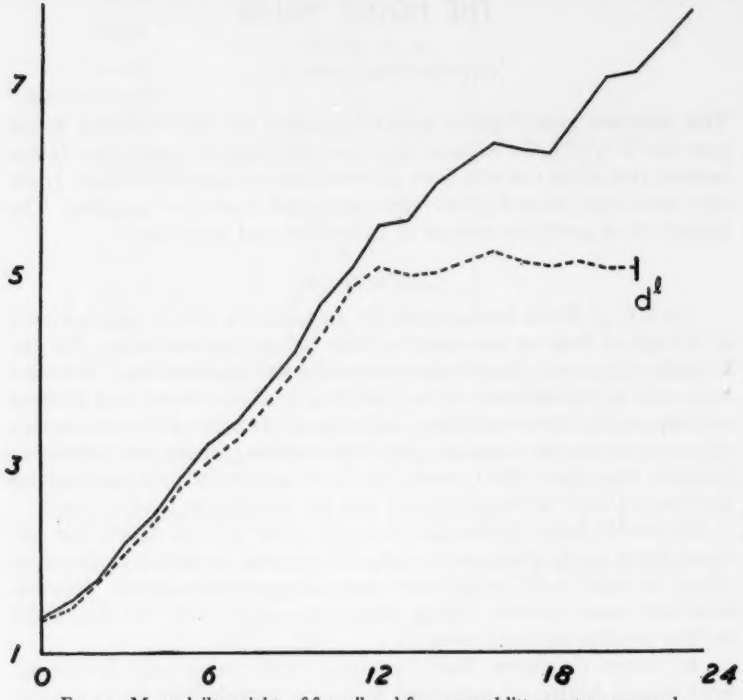


FIG. 1.—Mean daily weights of four d^1 and four normal litter-mates compared.

limb movements are generally less violent than when on a smooth surface.

Death ensues at about three weeks, preceded by great weakness and emaciation. Mutants have been kept alive for over a fortnight longer by transfer to a foster-mother before the age of weaning or by leaving plenty of wet food within easy reach. But the dilute lethals roll helplessly in the wet mash so that their fur is soon matted and foul.

WEIGHT

There was no significant difference in the birth-weights of normal and mutant, the mean weights of forty-nine normals being $1{\cdot}41 \pm 0{\cdot}03$ grams and of twenty-nine mutant litter-mates $1{\cdot}45 \pm 0{\cdot}04$ grams. At eleven days, normals were heavier on the average—$4{\cdot}98 \pm 0{\cdot}12$ grams against $4{\cdot}60 \pm 0{\cdot}22$ grams for d^1—but the difference was not quite sig-

nificant ($t=1{\cdot}73$, $n=32$ and $0{\cdot}1>P>0{\cdot}05$). From twelve days on, growth-curves of lethal and normal diverge rapidly, as figs. 1 and 2 show. The weights of affected animals may remain fairly stationary, as in fig. 1, or they may rise slowly and then fall rapidly, as fig. 2 shows. The falling-off from the normal growth pattern is presumably due to

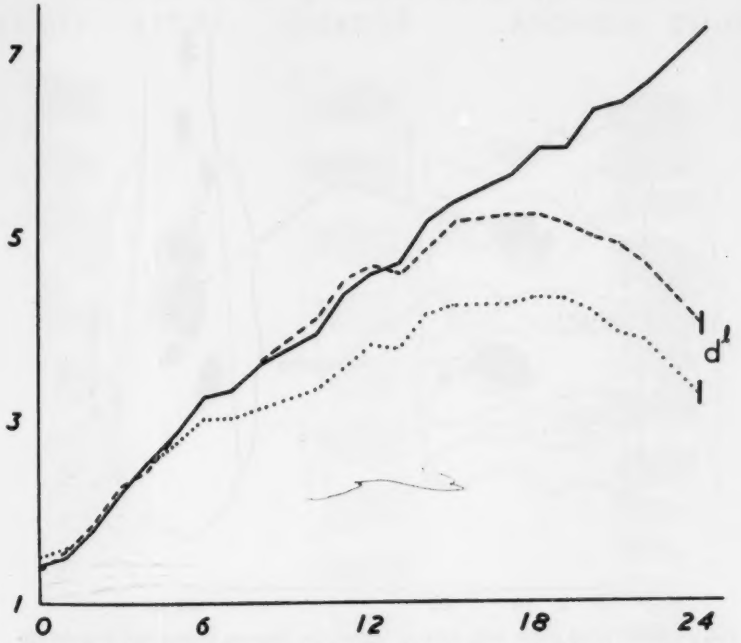


FIG. 2.—Individual growth-curves of two dilute lethals compared with the mean growth-curve for five normal litter-mates.

the great difficulty with which the mutants move around to reach food and water.

HAIR

The action of d^1 has been observed on a black, brown and yellow background. It dilutes all these fur-colours to the same extent as Maltese dilution (d): normal dilute black and brown mice have been observed in the same litters as the corresponding dilute lethals and appear identical in colour.

Preparations of d^1 hairs have been made by the method of E. S. Russell (1946). As in d hairs (Russell, 1949) there is intracellular clumping of pigment granules and irregularities of pigment distribution in and near the root-bulb, shown in fig. 3.

In black hair (fig. 4), the individual granules in medullary cells cannot be distinguished. The medullary cells of both d and d^1 mice

are very irregular and in both there has been some intercellular deposition of pigment to form very large clumps, as described by Russell (1949) for Maltese dilution. Cortical granules are lacking in the section of d^l hair shown, but this is not true for the whole length of the hair. But the numbers of cortical granules in equivalent regions of hairs of each dilute genotype have not been compared in actual counts.

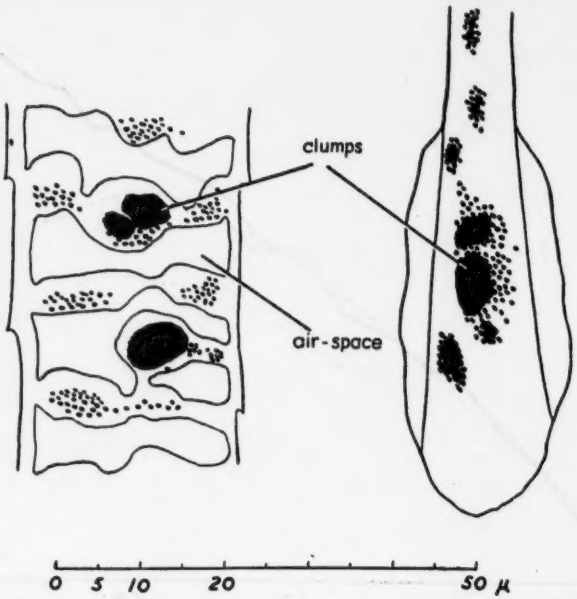


FIG. 3.—Dilute lethal yellow hair, showing intracellular pigment clumps and irregular distribution of pigment in the root. Camera lucida drawing.

OTHER INVESTIGATIONS

Under the age of ten days there was no difference in the response of normals and mutants to position change, when suspended by the tail. In both there was dorsiflexion of the spine, raising of the head and protraction and extension of the fore-limbs. Later, however, the lethal tends to start a convulsive fit when held in this position. Mutants over a fortnight old, which are almost completely incapable of voluntary movement on land, are nevertheless able to swim vigorously. But instead of making straight for the shore, like normals, they tend to go round in circles, recalling their motion when on their sides on land. No abnormalities of the inner ear were found when a histological examination of this organ was made.

An examination of brain sections has revealed no gross defects. In particular, the cerebellum appears to be structurally similar to that of a normal litter-mate, with all its cell-layers intact.

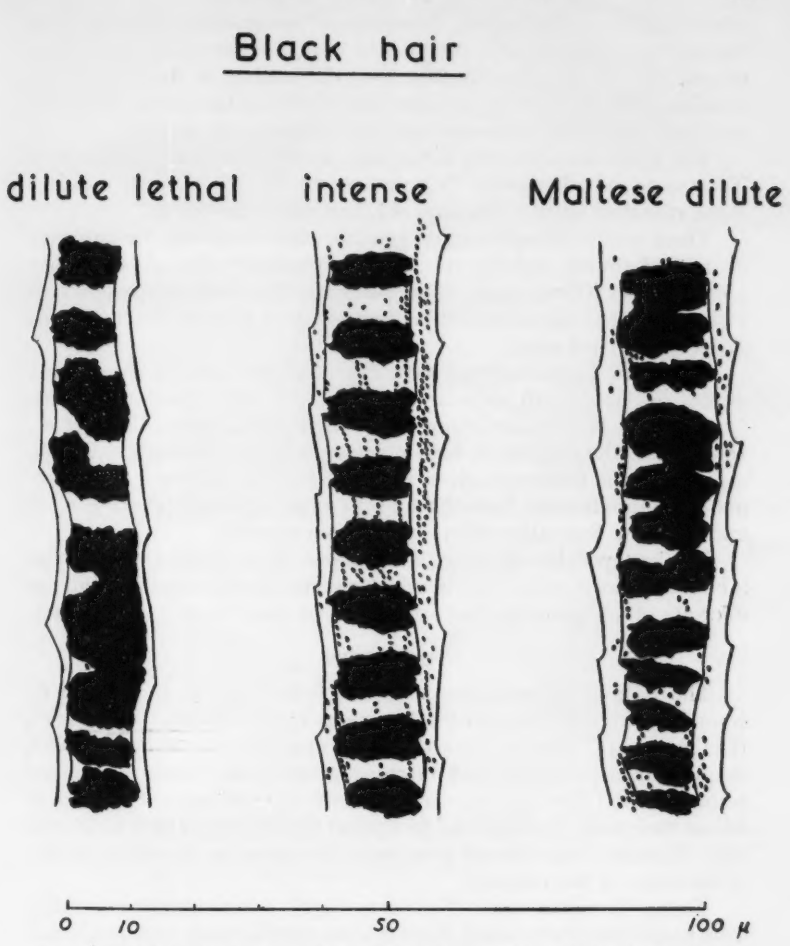


FIG. 4.—Black hair, showing intercellular pigment clumps and irregularity of cells in *d* and d^l. Camera lucida drawing.

SEGREGATION

In C 57 Black sibships segregating for d^l and containing fifteen or more classifiable young, the ratio of normals to mutants was 130 : 34, or 20·7 per cent d^l. Disregarding the slight degree of selection in these data, one would expect 25·0 per cent recessives; the observed ratio was in reasonable agreement with this expectation ($\chi^2 = 1{\cdot}59$, $n = 1$ and $P = 0{\cdot}21$).

Because of the close resemblance of the mutant's hair to that of a Maltese dilute phenotype, the gene was tested for allelomorphism against

dilute and its mimic leaden. None out of twenty-nine offspring from the matings of known heterozygotes to leaden females had diluted fur-colour. But of sixty-five offspring from the matings to short-ear dilute females, thirty-five, or 54 per cent, had a diluted fur-colour, in agreement with the 1 : 1 ratio expected with allelomorphism.

The F_1 dilutes were very fertile and perfectly normal in behaviour. They were mated together in pairs, producing 281 offspring. All of these, classified when a few days old, had dilute fur-colour.

These results strongly suggest genuine allelomorphism, though they do not, of course, entirely rule out the possibility that *d* and d^l are pseudo-alleles (Glass, 1949). If the two genes do in fact occupy different loci, the upper limit of recombination (on the 5 per cent level of significance) is 1·1 per cent.

The three F_2 phenotypes were found in the ratio 59 short-eared dilutes (*se d*/*se d*) : 116 long-eared dilutes (*se d*/ + d^l) : 70 dilute lethals (+ d^l / + d^l). The loci for *se* and *d* are only 0·2 cM. apart (Castle *et al.*, 1936) so all the animals in each phenotype group can fairly safely be assigned to the genotypes given in brackets. The observed ratio does not differ significantly from the 1 : 2 : 1 ratio expected ($\chi^2 = 1{\cdot}7$, n = 2 and P = 0·4). Sex-ratios differed little from equality.

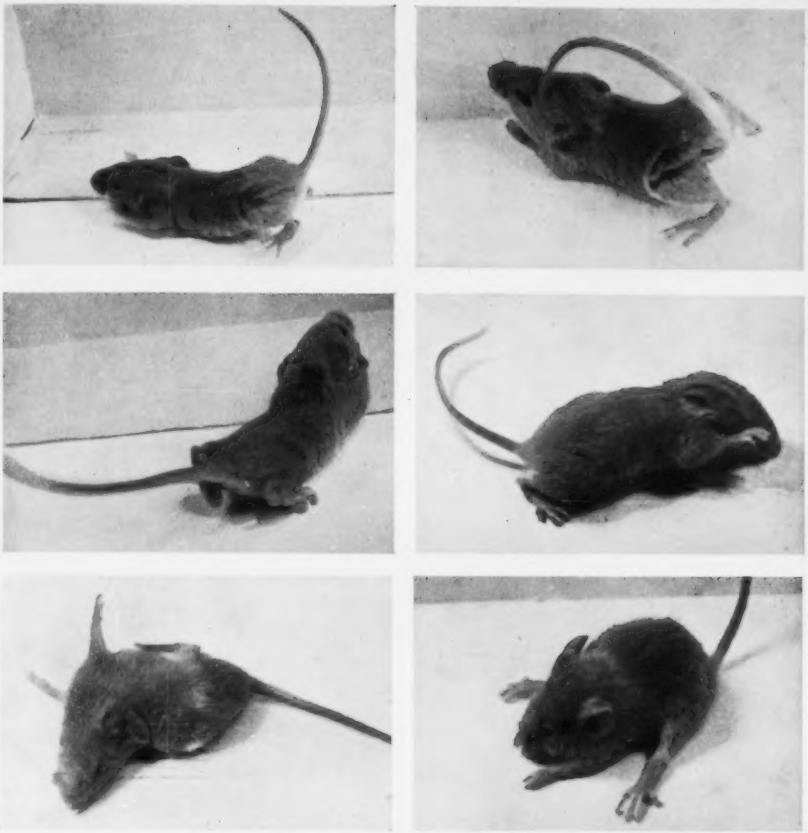
The juxtaposition of *se* and *d* loci allows dilute lethal to be kept as a balanced stock, *se* + / + d^l, in which practically all long-eared intense mice are of this genotype and are used to produce the next generation.

DISCUSSION

The pattern of behaviour of dilute lethals has some features in common with other neurological mutants in the mouse. Jittery mice (DeOme, 1945) show a marked lack of muscular co-ordination, with frequent over-balancing and lurching movements. Later they have convulsive fits; they also appear to suffer from opisthotonus when they fall on their sides, for they tend to support themselves on their head and tail. Harman (1950) found polycystic alterations in the white matter of the brain of this mutant.

Young Trembler mice (Falconer, 1951), when stimulated, suffer from convulsive fits in which there is some opisthotonus, and occasional vigorous up and down movements of the fore-limbs. But the characteristic rapid trembling movements of head and neck in the adult and the hyperextension of the limbs found throughout life have no counterpart in dilute lethal. With due precautions, most Tremblers pass out of their critical convulsive stage and reach adult life. But even in those dilute lethals which survived to five weeks there was no sign of any abatement in the severity of the symptoms.

Castle, Gates, Reed and Law (1936) have presented evidence to show that *d* is associated with an increased body-weight of about 2 per cent when compared with intense litter-mates, as well as associated changes in tail-length, etc. No attempt has yet been made to see if the compound d/d^l has a similar effect. Since *se* reduces adult size (Law,



PLATE—Dilute lethal's behaviour. *a* and *b*, Typical postures during fit, with marked opisthotonus and fore-limbs beating up and down. *c*, Hind-limbs involved in convulsions, with bouncing movement. *d*, Later stage, no opisthotonus. *e*, Lying on side and attempting to right itself. *f*, Huddled-up posture after fit.

1938) one would not be able to use this gene as a marker and the genotypes of the dilute animals would have to be found by progeny tests.

This mutant adds another item to the now lengthy list of pleiotropic genes in the mouse with major effects besides those on fur-colour. In none of these has the connexion between the colour changes and other effects been elucidated. Other members of the group with pleiotropic neurological effects are pallid and varitint-waddler. The former dilutes fur- and eye-colour and also often leads to absence of otoliths (Lyon, 1951); the latter combines spotting and dilution with circling, head-tossing and deafness, also epileptiform fits in the homozygote (Cloudman and Bunker, 1945).

SUMMARY

1. Dilute lethal (symbol d^l) is a new recessive mutation in the mouse. Affected animals suffer from neurological symptoms, which include clonic convulsions with opisthotonus and ataxia, which lead to death soon after weaning.

2. Evidence is presented to show that this gene is an allele of Maltese dilution.

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Note added in proof.—W. L. Russell, in his paper " X-ray-induced mutations in mice " (*Cold Spr. Harb. Sym. quant. Biol.* 1951. *16,* 327-336) reports the occurence of two induced and one spontaneous mutation at the *D* locus, which seems to be indistinguishable from dilute lethal, both in their action on fur and on behaviour.

A STUDY OF ULTRAVIOLET INDUCED LETHAL MUTATIONS IN *DROSOPHILA MELANOGASTER**

IRWIN I. OSTER
Indiana University, Bloomington, Indiana

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THE term lethal mutation as used in connexion with the genetics of *Drosophila* denotes a heritable change which causes the death of the individual before maturity is reached. Since the fly undergoes a complete metamorphosis, the lethal effect can be exerted during any of the three major stages (*i.e.* embryo, larva, pupa) prior to the hatching of the adult fly.

Previously Medvedev (1938), in a developmental analysis of 30 cases of sex-linked lethals that had arisen spontaneously and that showed no apparent changes in their salivary gland chromosomes, found that 15 exerted their lethal effect during the pupal stage. (It is not stated to what extent the possibility of allelism among these lethals had been ruled out but they were of independent origin.) In 1939 he reported that of 12 of these same spontaneous sex-linked lethals which killed in some pre-pupal stage, 2 caused death during the embryonic period, 9 during the 1st and 1 during the 2nd larval instar. Five others among the total of 30 were observed to die in the third larval instar, at about the time of pupation. He interpreted this apparent grouping as showing three " sensitive periods "—in the embryo, the first instar, and the time of pupation, respectively. From a subsequent analysis of another group of 18 sex-linked lethals (composed of 8 spontaneous and 10 ultraviolet-induced lethals which, however, were not examined cytologically) in which he found that 1 caused death during the embryonic period, 8 during the 1st and 1 during the 3rd larval instar, and 8 during the pupal period, Medvedev (1939*b*) concluded that " the inability of further development of the lethal larvae was connected with the disturbance of such processes as moulting and pupation in some of the cases, at least ".

Poulson (1937, 1940, 1945) found that deficiencies usually cause embryonic death, as might be expected in view of their involving multilocus losses, since (1) the earliest effect would be the one detected and since (2) some effects would be synergistic and therefore tend, when in combination, to cause lethality at a time at which, separately, they would have been merely detrimental. Hadorn (1948) tabulated the stages of death for 38 lethals reported on in previous work of various authors. Death was found to occur at various stages and Hadorn inter-

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prets the results as showing a clustering of deaths at 4 periods: the early embryo, the period just before or after hatching from the egg case, that just before or after pupa formation, and that just before or after eclosion from the pupa case. He regards the "*phases of high developmental activity*" as those especially subject to the action of lethals. However, since many of the lethals used by him were known to be allelic, and many were deficiencies, the collected data which he presents are such as to provide little evidence of what the relative frequency of death at different stages would be for gene mutations.

During the past five years a large number of stocks containing lethal mutations which have occurred spontaneously or have been induced by X-rays, neutrons, or ultraviolet light have been accumulated by Dr H. J. Muller and his co-workers at Indiana University. It was desired to examine the lethals having these four origins with regard to the time during development that they kill and the morphological abnormalities that they induce. Those mutations entailing no detectable structural changes in the chromosomes, are especially well suited for throwing light on the action of individual genes on the physiological processes involved in development. Most ultraviolet lethals fall in this category. These may be referred to in a non-committal way as due to "point mutation" but the great majority are presumably results of gene mutation. This paper reports the results of a developmental study of a group of these point mutational ultraviolet-induced lethals.

METHODS

We had at our disposal a collection of sex-linked lethals which had been obtained by ultraviolet irradiation of *Drosophila melanogaster* spermatozoa in experiments by J. T. McQuate (1951). These had been localised by McQuate in linkage tests and found to show no abnormality in the frequency of crossing over. They had then been determined by Dr J. I. Valencia by cytological examination of the appropriate portion of the salivary gland chromosomes to entail no visible structural changes of the X chromosome (Valencia and McQuate, 1951).

The lethals were contained in balanced stocks, with the lethal X chromosome distinguished from its homologue by having the inversions (In) designated and the marker yellow body (y), and white eye (w), as well as by being normal with regard to Bar eye (B). The composition of the X chromosome of all these stocks was as follows, except that the locus of the lethal, *l*, could—according to the case—be anywhere in the X chromosome.

$$\frac{l\ \mathrm{y\ w\ In49}}{\mathrm{sc^{s1}\ B\ InS}}\,♀ \times \frac{\mathrm{sc^{s1}\ B\ InS}}{\qquad}\,♂$$

The stock is balanced because females homozygous for sc^{s1} are sterile.

These stocks allow the male larvae with the lethal chromosome to be recognised by their light mouth parts (due to y) and their colourless Malpighian tubes (due to w) (Bridges and Brehme, 1944). For pur-

poses of comparison of larvae of the same age, a sample of at least 50 lethal-containing male larvae of each stock, derived from eggs laid on a given day, were transferred to a culture by themselves, and watch was kept upon them thereafter, several of them being examined microscopically each day. The preliminary findings, reported along with our interpretation, in an abstract in *Drosophila Information Service* (Oster, 1951), were checked by subsequent examinations. Death in embryonic stages was shown by the presence of brownish eggs and the absence of y w larvae. When death occurred in larval stages, the instar to which viability extended could be determined by the morphology of the spiracles (Rühle, 1932). The moribund larvae and other stages were observed and dissected microscopically in insect Ringer's solution.

OBSERVATIONS AND RESULTS OF EXPERIMENTS*

Fifty-five of the ultraviolet-induced lethals involving " point mutations " only have been subjected to the above type of study. The numbers of lethals which become effective during the different stages of development are listed in table 1:

TABLE 1

The stages of development during which the different lethal mutations act

Embryonic stages			Larval instars			Pupal stages		
early	middle	late	1st	2nd	3rd	early	middle	late
0	0	7	27	0	9	9	0	3
								Total 55

This table indicates only the most advanced stage to which the particular lethals could survive. Since some of the deleterious effects of the mutants must have been manifested earlier in development some of the individuals must have died before the most critical stage was reached. Usually, however, there was no conspicuous dying off of the lethal larvae previous to their most critical stage.

Of these 55 stocks, 47 showed no morphological abnormalities as determined by total-mount microscopic inspection of the dead or dying stage at a 100× magnification, and dissection of them at 30×, while eight exhibited some visible derangement, which might be related to the cause of death. The symbol, the designation, the locus in chromosome 1, and the characteristic visible effects of these eight lethals are as follows:

*l*jl: lethal, jawless; 14. Dies during the 1st larval instar, mouth parts poorly formed and sometimes absent.

*l*ml: lethal, melanoma-like; 1. Dies during the 3rd larval instar, larvae at time of death have internal black and brown melanotic masses (usually 1 or 2, sometimes as many as 10); may represent a malignancy.

*l*mt: lethal, midget; 2.5. Dies as undersized 3rd instar larvae.

* After completion of the work reported herein it was learned through a personal communication that M. T. M. Rizki has independently carried out a similar study on another species of *Drosophila* and that his data (which we have not yet had an opportunity to see) are similar, so far as spontaneous lethals are concerned, to those reported here for ultraviolet.

*l*rr: lethal, ring gland rudimentary; 0.3. Dies during the 3rd larval instar, reduction of the ring gland with associated failure to undergo the 3rd moult, otherwise normal; often lives for 15-30 days but does not become a giant.

*l*te: lethal, tracheae enlarged; 0.3. Dies during the 3rd larval instar, main tracheal tubes greatly enlarged, sometimes lacks functional posterior spiracles.

*l*tl: lethal, tracheae lacking; 59. Dies during the 1st larval instar, no evidence of main tracheal tubes although small side branches are present.

*l*tr: lethal, tracheae ramified; 56. Dies during the 1st larval instar, main tracheal tubes are thick and have very many side branches.

*l*ts: lethal, tracheae stretched; 8. Dies during the 1st larval instar, very large larvae for this stage, all the tracheal tubes are very thin, suggesting that they do not grow at the same rate as the larvae and thus become stretched.

The reason why such a large proportion of the visible changes involved the tracheae is probably because these organs are so conspicuous and have such a complicated, definitely expressed morphology, alterations in which are readily observed.

DISCUSSION

A consideration of the data indicates that there is a clustering of mortality at the end of embryonic, at the beginning and end of larval life, and the beginning and end of pupal life. These are all stages where many newly developed parts and processes become functionally active for the first time. The lethal gene may have influenced development earlier but the effect on survival could have been little if any until the affected part or process was called on to participate in some vital physiological function. This is well illustrated in stock *l*rr. The lethal larvae of this stock remain small and live 15 days or more as 3rd instar larvae but are unable to pupate because of a reduced ring gland. However, the ring gland deficiency already was determined during the 1st and 2nd larval instars by a failure of the gland's cells to enlarge.

Although the greatest developmental changes of all take place in the early and middle embryonic period relatively few lethals were found to act then, doubtless because functioning of the new parts is not yet active and so they are not yet important for life. The successful development and functioning of the early and middle stage embryo are, to a large extent, dependent on gene products already elaborated during the previous diploid stage when the lethal mutation, being present along with its normal allele, was not exerting its deleterious effects. On the other hand, nearly half of all the lethals express themselves during the 1st larval instar, when the manifold physiological (nervous, secretory, muscular, nutritive, circulatory, etc.) processes characteristic of larval life are required. Similarly, during the end of larval and the beginning of pupal life, new and different physiological processes must enter in and thus many lethals are found which express themselves then. The fact that no lethals were found which act mainly during the 2nd instar is to be expected, since that period is not characterised by radically new physiological functions but only by slight morphological changes.

In addition to the above cases of gene mutation one case of ultraviolet-induced deficiency (bands 15A2-3 to 15C4-5 according to J. I.

Valencia) and one of translocation were studied similarly. The former proved to be lethal to the embryo, like most other deficiencies, although death occurred at a late embryonic stage. The latter caused death during the 1st larval instar.

SUMMARY

1. The results are presented of a developmental analysis of 55 ultraviolet-induced sex-linked lethals due to point mutation, presumably involving in each case the alteration of an individual gene.

2. The time during development when the lethals kill and the morphological abnormalities induced by them are described. Of the eight which caused detected abnormalities, four affected the morphology of the tracheae and one gave rise to melanotic tumours.

3. There is a clustering of mortality at the end of embryonic life, at the beginning and end of larval life, and at the beginning and end of pupal life. This clustering is interpreted as being caused by the fact, not that development *per se* is more active at these times, but that these are the periods in which many physiological functions new to the individual come into active operation for the first time in its life.

Acknowledgment.—I wish to express my sincere apppreciation to Dr H. J. Muller for his helpful interest during the course of the work, and for his proposal of the interpretation above offered.

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REVIEWS

THE MITOTIC CYCLE. The cytoplasm and nucleus during the interphase and mitosis. Arthur Hughes, M.A., Ph.D., 1952. London: Butterworths Sci. Publns. Pp. i-viii, 1-132. 35s.

This book is concerned with the animal and plant cell in relation especially to mitosis. Dr Hughes has done a conscientious piece of work. So also have his two collaborators, Dr Swann and Dr Waymouth. Dr Hughes approaches the problem from the point of view of his own excellent studies on living cell division and of the physical and chemical properties of the cell constituents. He skirts the fringe of the genetical and physiological sides of the problem. He comes nearer to them, however, than any of his predecessors in this field for he is interested in the nucleus and he gives it a central position in the cell. Why he gives it this position however is not clear. He often refers, for example, to meiosis but what happens at meiosis, and what experiments have been applied to meiosis, he does not tell us. Nor does he realise what he has missed.

Dr Hughes appreciates that it is somehow unsatisfactory to have discovered bodies and processes so rich in form but so poor in function. The thousand references which he and his collaborators painstakingly quote leave him, as he explains, knowing very little about the cell. At the end the reader may also feel that he knows more about the authors and the papers than about the things they discuss. This state of ignorance is nothing new. It must be very common among those who teach this subject. But if it is to be the basis of writing a book, even a specialist's book, the author should indicate what the little is that he knows or believes. He should explain what foundations he is attempting to build on. This Dr Hughes omits to do.

Bound up with these accustomed difficulties of theory, Dr Hughes encounters a novel difficulty of technique. The method of dividing a book into sections of a similar length but a different subject matter, was no doubt invented for epic poetry. But it has proved useful in all kinds of writing. In science, it has sometimes enabled authors to reach positive conclusions—even in regard to the cell. But Dr Hughes dispenses with this helpful practice. One of his six chapters fills nearly half the book and most of his chapters cover, not the same papers of course, but the same things. He has thus failed to learn what he might have learnt from writing this book. He has also made it into a serious exercise for the reader who wishes to do better.

C. D. DARLINGTON

EUGENICS: Galton and After. C.P. Blacker, M.C., G.M., M.A., M.D., F.R.C.P., 1952. London: Duckworth. Pp. 349. 25s.

The importance of Galton in the founding of Genetics is not generally understood. This book provides the evidence of what he did, and of its consequences down to the present day, in a convenient form for the student of heredity. The bibliography of Galton's works seems to be complete except for the most curious and inaccessible of all, the popular article in Frazer's Magazine in which he first distinguished between one-egg and two-egg

twins: it was in 1875, the year in which Hertwig discovered the nature of fertilisation.

INQUIRIES INTO HUMAN FACULTY AND ITS DEVELOPMENT. Sir Francis Galton. 1883. Reprinted 1951. London: Dent & Sons. Pp. 261. 7s. 6d.

THREE MEMOIRS. Supplementary pamphlet. Sir Francis Galton. 1951. London: Cassell. Pp. 23. 1s. 6d.

Galton's Human Faculty first appeared in 1883. It represents his pioneer contributions to the foundation of biometry, genetics and psychology.

This book is the edition of the Everyman library now reprinted with a supplementary " Three Memoirs " being the part previously purged from it. It is published for the Eugenics Society by Messrs Dent & Sons through Messrs Cassell.

A HUNDRED YEARS OF BIOLOGY. Ben Dawes, D.Sc., 1952. London: Duckworth. Pp. 429. 30s.

An historical abstract sometimes distorted by condensation but with a compendious index and a bibliography of 1800 references. Dr Dawes is Reader in Zoology, King's College, London.

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